...TENT COOPERATION TRE... Y

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

KOKULIS, Paul, N.
Pillsbury Winthrop LLP
1600 Tysons Boulevard
McLean, VA 22102
ETATS-UNIS D'AMERIQUE

Date of mailing (day/month/year)
08 February 2002 (08.02.02)

Applicant's or agent's file reference
LEJA 067 PCT

IMPORTANT NOTIFICATION

International application No.
PCT/US00/21732

International filing date (day/month/year)
10 August 2000 (10.08.00)

1. The following indications appeared on record concerning:

[X] the applicant [X] the inventor [ ] the agent [ ] the common representative

Name and Address
ZITZMANN, N. & BUTTERS, T.,D.
PLATT, F.,M. & CARROUEE, S.
JACOB, G.,S. & PICKER, D.,H.
FLEET, G.,W.J. & DWEK, R.,A.
DURANTEL, D. & MEHTA, A.
BLOCK, T.,M.

State of Nationality | State of Residence

Telephone No.

Facsimile No.

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address

State of Nationality | State of Residence

Telephone No.

Facsimile No.

Teleprinter No.

3. Further observations, if necessary:
The status of the above-mentioned applicants/inventors has been changed from applicant/inventor for all designated States to applicant/inventor for US only.

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer
François BAECHLER

Telephone No.: (41-22) 338.83.38

ATENT COOPERATION TR. .TY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

KOKULIS, Paul, N.
Pillsbury Winthrop LLP
1600 Tysons Boulevard
McLean, VA 22102
ETATS-UNIS D'AMERIQUE

Date of mailing (day/month/year)
14 November 2001 (14.11.01)

Applicant's or agent's file reference
LEJA 067 PCT

IMPORTANT NOTIFICATION

International application No.
PCT/US00/21732

International filing date (day/month/year)
10 August 2000 (10.08.00)

1. The following indications appeared on record concerning:
[X] the applicant [X] the inventor [ ] the agent [ ] the common representative

Name and Address

State of Nationality | State of Residence

Telephone No.

Facsimile No.

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:
[X] the person [X] the name [X] the address [X] the nationality [X] the residence

Name and Address
BLOCK, Timothy, M.
90 Foxcroft Drive
Doylestown, PA 18901
United States of America

State of Nationality: US | State of Residence: US

Telephone No.

Facsimile No.

Teleprinter No.

3. Further observations, if necessary:
Additional applicant/inventor for all designated States.

4. A copy of this notification has been sent to:
[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

Authorized officer
François BAECHLER
Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TRE. TY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

KOKULIS, Paul, N.
Pillsbury Winthrop LLP
1600 Tysons Boulevard
McLean, VA 22102
ETATS-UNIS D'AMERIQUE

Date of mailing (day/month/year) 14 November 2001 (14.11.01)	
Applicant's or agent's file reference LEJA 067 PCT	**IMPORTANT NOTIFICATION**
International application No. PCT/US00/21732	**International filing date** (day/month/year) 10 August 2000 (10.08.00)

1. The following indications appeared on record concerning:

[X] the applicant [X] the inventor [ ] the agent [ ] the common representative

Name and Address	State of Nationality	State of Residence
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[X] the person [X] the name [X] the address [X] the nationality [X] the residence

Name and Address	State of Nationality	State of Residence
MEHTA, Anand 335 Indiantown Road Landenberg, PA 19350 United States of America	US	US
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:
Additional applicant/inventor for all designated States.

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer François BAECHLER
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

ATENT COOPERATION TR. TY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

KOKULIS, Paul, N.
Pillsbury Winthrop LLP
1600 Tysons Boulevard
McLean, VA 22102
ETATS-UNIS D'AMERIQUE

Date of mailing (day/month/year)
14 November 2001 (14.11.01)

Applicant's or agent's file reference
LEJA 067 PCT

IMPORTANT NOTIFICATION

International application No.
PCT/US00/21732

International filing date (day/month/year)
10 August 2000 (10.08.00)

1. The following indications appeared on record concerning:
[X] the applicant [X] the inventor [ ] the agent [ ] the common representative

Name and Address

State of Nationality | State of Residence

Telephone No.

Facsimile No.

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:
[X] the person [X] the name [X] the address [X] the nationality [X] the residence

Name and Address
DURANTEL, David
20, avenue du Maréchal de Lattre
de Tassigny
F-13200 Arles
France

State of Nationality: FR | State of Residence: FR

Telephone No.

Facsimile No.

Teleprinter No.

3. Further observations, if necessary:
Additional applicant/inventor for all designated States.

4. A copy of this notification has been sent to:
[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

Authorized officer
François BAECHLER
Telephone No.: (41-22) 338.83.38

ATENT COOPERATION TRI TY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

KOKULIS, Paul, N.
Pillsbury Winthrop LLP
1600 Tysons Boulevard
McLean, VA 22102
ETATS-UNIS D'AMERIQUE

Date of mailing (day/month/year)
14 November 2001 (14.11.01)

Applicant's or agent's file reference
LEJA 067 PCT

IMPORTANT NOTIFICATION

International application No.
PCT/US00/21732

International filing date (day/month/year)
10 August 2000 (10.08.00)

1. The following indications appeared on record concerning:
[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address

State of Nationality | State of Residence

Telephone No.

Facsimile No.

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:
[X] the person [X] the name [X] the address [X] the nationality [X] the residence

Name and Address
SYNERGY PHARMACEUTICALS, INC.
Suite 450
Two Executive Drive
Somerset, NJ 08873
United States of America

State of Nationality: US | State of Residence: US

Telephone No.

Facsimile No.

Teleprinter No.

3. Further observations, if necessary:
Additional applicant for all designated States except US.

4. A copy of this notification has been sent to:
[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

Authorized officer
François BAECHLER
Telephone No.: (41-22) 338.83.38

ATENT COOPERATION TRI TY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

KOKULIS, Paul, N.
Pillsbury Winthrop LLP
1600 Tysons Boulevard
McLean, VA 22102
ETATS-UNIS D'AMERIQUE

Date of mailing (day/month/year)
14 November 2001 (14.11.01)

Applicant's or agent's file reference
LEJA 067 PCT

IMPORTANT NOTIFICATION

International application No.
PCT/US00/21732

International filing date (day/month/year)
10 August 2000 (10.08.00)

1. The following indications appeared on record concerning:
[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address

State of Nationality | State of Residence

Telephone No.

Facsimile No.

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:
[X] the person [X] the name [X] the address [X] the nationality [X] the residence

Name and Address
THOMAS JEFFERSON UNIVERSITY
1020 Walnut Avenue
Philadelphia, PA 19107
United States of America

State of Nationality: US | State of Residence: US

Telephone No.

Facsimile No.

Teleprinter No.

3. Further observations, if necessary:
Additional applicant for all designated States except US.

4. A copy of this notification has been sent to:
[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer
François BAECHLER

Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994)

004451443

. ATENT COOPERATION TRL .TY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

KOKULIS, Paul, N.
Pillsbury Winthrop LLP
1600 Tysons Boulevard
McLean, VA 22102
ETATS-UNIS D'AMERIQUE

Date of mailing (day/month/year)
14 November 2001 (14.11.01)

Applicant's or agent's file reference
LEJA 067 PCT

IMPORTANT NOTIFICATION

International application No.
PCT/US00/21732

International filing date (day/month/year)
10 August 2000 (10.08.00)

1. The following indications appeared on record concerning:
[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address

State of Nationality | State of Residence

Telephone No.

Facsimile No.

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:
[X] the person [X] the name [X] the address [X] the nationality [X] the residence

Name and Address
THE CHANCELLOR, MASTERS, AND SCHOLARS OF THE UNIVERSITY OF OXFORD
University of Oxford
University Offices
Wellington Square
Oxford OX1 2JD
United Kingdom

State of Nationality: GB | State of Residence: GB

Telephone No.

Facsimile No.

Teleprinter No.

3. Further observations, if necessary:
Additional applicant for all designated States except US.

4. A copy of this notification has been sent to:
[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

Authorized officer
François BAECHLER
Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 22 June 2001 (22.06.01)	
International application No. PCT/US00/21732	**Applicant's or agent's file reference** LEJA 067 PCT
International filing date (day/month/year) 10 August 2000 (10.08.00)	**Priority date** (day/month/year) 10 August 1999 (10.08.99)
Applicant ZITZMANN, Nicole et al	

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International Preliminary Examining Authority on:

   08 March 2001 (08.03.01)

   [ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

   [ ] was not

   made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Odile ALIU

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

KOKULIS, Paul, N.
Pillsbury Winthrop LLP
1600 Tysons Boulevard
McLean, VA 22102
ETATS-UNIS D'AMERIQUE

Date of mailing (day/month/year) 14 November 2001 (14.11.01)	
Applicant's or agent's file reference LEJA 067 PCT	**IMPORTANT NOTIFICATION**
International application No. PCT/US00/21732	**International filing date** (day/month/year) 10 August 2000 (10.08.00)

1. The following indications appeared on record concerning:

☐ the applicant ☐ the inventor ☒ the agent ☐ the common representative

Name and Address	State of Nationality	State of Residence
KOKULIS, Paul, N. Pillsbury Madison & Sutro, LLP 1100 New York Avenue, N.W. Washington, DC 20005 United States of America		
	Telephone No. 202 861 3000	
	Facsimile No. 202 822 0944	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☐ the name ☒ the address ☐ the nationality ☐ the residence

Name and Address	State of Nationality	State of Residence
KOKULIS, Paul, N. Pillsbury Winthrop LLP 1600 Tysons Boulevard McLean, VA 22102 United States of America		
	Telephone No. (703) 905-2000	
	Facsimile No. (703) 905-2500	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

☒ the receiving Office
☐ the International Searching Authority
☒ the International Preliminary Examining Authority
☐ the designated Offices concerned
☒ the elected Offices concerned
☐ other:

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer François BAECHLER
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994) 004451110

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
LEJA 067 PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 00/ 21732	10/08/2000	10/08/1999
Applicant		
ZITZMANN, Nicole et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[ ] the text is approved as submitted by the applicant.

[X] the text has been established by this Authority to read as follows:

LONG CHAIN N-ALKYL COMPOUNDS AND OXA-DERIVATIVES THEREOF AND USE AS ANTIVIRAL COMPOSITIONS

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[X] None of the figures.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1,2,4-22,26-32,36,37

Present claims 1,2,4-11,26-31,36,37 relate to compounds which are actually not well defined, since the definition specifies only part of their chemical structure: N-C8-C16 alkyl group or oxa-substituted derivative thereof.
The use of these parameters in the present context is considered to lead to a lack of clarity within the meaning of Article 6 PCT. It is impossible to compare the parameters the applicant has chosen to employ with what is set out in the prior art.
Present claims 12-22,26-32 relate to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search is impossible.
The lack of clarity is such as to render a meaningful complete search impossible. Consequently, the search has been restricted to the compounds described in claims 3,23-25,33-35.

Claims searched completely: 3,23-25,33-35.
Claims not searched: 1,2,4-22,26-32,36,37.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/21732

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/435 A61K31/44 A61K31/445 A61K31/40 A61K31/195
A61K31/13 A61P31/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
CHEM ABS Data, EPO-Internal, WPI Data, PAJ, SCISEARCH, EMBASE, MEDLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 35685 A (DWEK RAYMOND A ;SEARLE & CO (US); BLOCK TIMOTHY M (US); JACOB GARY) 20 August 1998 (1998-08-20)	35
A	page 12, line 8 - line 30	3,23-25, 33-35
X	EP 0 445 098 A (SEARLE & CO) 4 September 1991 (1991-09-04)	35
A	example 4 page 2, line 31 -page 3, line 18	3,23-25, 33-35
X	US 5 310 745 A (PARTIS RICHARD A ET AL) 10 May 1994 (1994-05-10)	35
A	column 4, line 33 column 1, paragraph 3	3,23-25, 33-35

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 February 2001	28/02/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Bonzano, C

4

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X A	WO 99 24401 A (SEARLE & CO ;JACOB GARY S (US)) 20 May 1999 (1999-05-20) claims 1,12	33 3,23-25, 33-35
A	VAN DEN BROEK, L. A. G. M. ET AL: "Chemical modification of aza sugars, inhibitors of N-glycoprotein- processing glycosidases and of HIV-I infection. Review and structure-activity relationships" RECL. TRAV. CHIM. PAYS-BAS (1993), 112(2), 82-94 , XP000979525 figure 6 table 3 page 90, column 2, paragraph 2 page 91, column 2, paragraph 3 - paragraph 7	3,23-25, 33-35
A	VAN DEN BROEK, L. A. G. M. ET AL: "Synthesis of oxygen-substituted N-alkyl 1-deoxynojirimycin derivatives: aza sugar.alpha.-glucosidase inhibitors showing antiviral (HIV-1) and immunosuppressive activity" RECL. TRAV. CHIM. PAYS-BAS (1994), 113(11), 507-16 , XP000979526 page 508, column 1 figures 2,N7	3,23-25, 33-35
A	ESPOSITO, ANNAMARIA ET AL: "Synthesis of amphiphilic polyhydroxylated pyrrolidines as potential glycosidase inhibitors" TETRAHEDRON LETT. (1998), 39(36), 6543-6546 , XP004132541 figures 5-11 page 6543, line 1 - line 11	3,23-25, 33-35
A	EP 0 367 747 A (SEARLE & CO) 9 May 1990 (1990-05-09) examples 11,12 claims 1,13 page 2, line 1 - line 25	3,23-25, 33-35

INTERNATIONAL SEARCH REPORT

[redacted]mation on patent family members

[redacted]ernational Application No
PCT/US 00/21732

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9835685 A	20-08-1998	AU 6169298 A	08-09-1998
		CN 1251993 T	03-05-2000
		EP 1007058 A	14-06-2000
EP 0445098 A	04-09-1991	CA 2036964 A	27-08-1991
		JP 5085935 A	06-04-1993
		US 5030638 A	09-07-1991
US 5310745 A	10-05-1994	US 5003072 A	26-03-1991
		US 5411970 A	02-05-1995
		US 5476859 A	19-12-1995
		AT 117678 T	15-02-1995
		CA 2002106 A,C	03-05-1990
		DE 68920819 D	09-03-1995
		EP 0367748 A	09-05-1990
		ES 2070929 T	16-06-1995
		GR 3015931 T	31-07-1995
		JP 2172975 A	04-07-1990
		JP 2907462 B	21-06-1999
		MX 9203547 A	01-09-1992
		US 5144037 A	01-09-1992
		US 5221746 A	22-06-1993
WO 9924401 A	20-05-1999	AU 1297399 A	31-05-1999
		EP 1030839 A	30-08-2000
EP 0367747 A	09-05-1990	US 4876268 A	24-10-1989
		US 4937357 A	26-06-1990
		US 4973602 A	27-11-1990
		AT 135346 T	15-03-1996
		CA 2002105 A,C	03-05-1990
		DE 68925940 D	18-04-1996
		DE 68925940 T	24-10-1996
		ES 2086323 T	01-07-1996
		GR 3019885 T	31-08-1996
		JP 2172972 A	04-07-1990
		JP 2840334 B	24-12-1998

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
LEJA 067 PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/21732	10/08/2000	10/08/1999
International Patent Classification (IPC) or national classification and IPC A61K31/00		
Applicant ZITZMANN, Nicole et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 1 sheet~~s~~.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
08/03/2001	11.12.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich T l. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Young, A Telephone No. +49 89 2399 7811

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/21732

I. Basis f the r port

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-31	as originally filed				
32	as received on	12/03/2001	with letter of	08/03/2001	

Claims, No.:

1-37	as originally filed

Drawings, sheets:

1/8-8/8	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 1-31.

because:

   ☒ the said international application, or the said claims Nos. with respect to IA relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 1, 2, 4-22, 26-32, 36 and 37.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack f unity f inv nti n

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:
see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☒ the parts relating to claims Nos. 1-6, 10-13 and 17-37 partially.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	3-4 and 23-25
	No:	Claims	1-2, 5-6, 10-13, 17-22 and 26-37
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-6, 10-13 and 17-37
Industrial applicability (IA)	Yes:	Claims	1-31 (see separate sheet) and 32-37
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
s e separat sh et

Re Item III:

Claims 1-31 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Item IV:

I. The subject-matter of independent claim 1 is already known in the art.
WO 98 35685 discloses N-substituted-1,5-dideoxy-1,5-imino-D-glucitol compounds (see claims 1 and 19) for the treatment of a hepatitis virus infection.
The requisite unity of invention (Rule 13.1 PCT) therefore no longer exists inasmuch as a technical relationship involving one or more of the same or cor- responding special technical features in the sense of Rule 13.2 PCT does not exist between the subject-matter of the following chemical groups related to claim 5:
N-alkylated piperidines, N-alkylated pyrrolidines, N-alkylated phenylamines, N- alkylated pyridines, N-alkylated pyrroles and N-alkylated amino acids.

II. Applicant explains himself in the description page 2 that HBV is a hepadnavirus - a DNA-containing virus and that HCV is a pestivirus - a RNA containing virus. Independant claim 1 reads a method of inhibiting morphogenesis of a pestivirus or a flavivirus. Dependant claim 7, 8, 9 and 30 refer to a method for inhibition of hepatitis B virus. Since hepatitis B virus is clearly nor a pestivirus neither a flavivirus, it cannot be understood how a method of inhibiting a pestivirus or a flavivirus would also solve the problem of inhibition of a hepatitis B virus.
Therefore the method of inhibition of hepatitis B virus is regarded nonunitary to the method of inhibiting morphogenesis of pestivirus or flavivirus.
In conclusion 12 different groups of inventions are identified in the application as listed below.
1. A method of inhibiting morphogenesis of a pestivirus or a flavivirus with an N-alkylated piperidine: Claims 1-6, 10-13, 17-37 all partially.
2. A method of inhibiting morphogenesis of a pestivirus or a flavivirus with an N-alkylated pyrrolidine: Claims 1-6, 10-13, 17-23, 26-32, 34, 36-37 all partially.
3. A method of inhibiting morphogenesis of a pestivirus or a flavivirus with an N-alkylated phenylamine: Claims 1-2, 4-5, 10-13, 16, 22-23, 26-32, 34, 36-37 all partially.
4. A method of inhibiting morphogenesis of a pestivirus or a flavivirus with an N-alkylated pyridines: Claims 1-2, 4-5, 10-13, 22, 26-32, 36-37 all partially.

5. A method of inhibiting morphogenesis of a pestivirus or a flavivirus with an N-alkylated pyrrole: Claims 1-2, 4-5, 10-13, 22, 26-32, 36-37 all partially.
6. A method of inhibiting morphogenesis of a pestivirus or a flavivirus with an N-alkylated amino acid: Claims 1-2, 4-5, 10-15, 22, 26-32, 36-37
7. A method for inhibition of hepatitis B virus with an N-alkylated piperidine: Claims 7-9, 11, 26-37 all partially
8. A method for inhibition of hepatitis B virus with an N-alkylated pyrrolidine: Claims 7-9, 11, 26-32, 34, 36-37 all partially.
9. A method for inhibition of hepatitis B virus with an N-alkylated phenylamine: Claims 7-9, 11, 26-32, 34, 36-37 all partially.
10. A method for inhibition of hepatitis B virus with an N-alkylated pyridine: Claims 7-9, 11, 26-32, 36-37 all partially.
11. A method for inhibition of hepatitis B virus with an N-alkylated pyrrole: Claims 7-9, 11, 26-32, 36-37 all partially.
12. A method for inhibition of hepatitis B virus with an N-alkylated amino acid: Claims 7-9, 11, 26-32, 36-37 all partially.

The examination will be carried out, according to Article 34 (3)(c), for the main invention, which is regarded the first defined invention.

Re Item V:

The following documents mentioned in the search report are considered the relevant state of the art:

D1: WO 98 35685 A (DWEK RAYMOND A ;SEARLE & CO (US); BLOCK TIMOTHY M (US); JACOB GARY) 20 August 1998 (1998-08-20)

D2: EP-A-0 445 098 (SEARLE & CO) 4 September 1991 (1991-09-04)

D3: US-A-5 310 745 (PARTIS RICHARD A ET AL) 10 May 1994 (1994-05-10)

D4: WO 99 24401 A (SEARLE & CO ;JACOB GARY S (US)) 20 May 1999 (1999-05-20)

D5: VAN DEN BROEK, L. A. G. M. ET AL: 'Chemical modification of aza sugars, inhibitors of N-glycoprotein- processing glycosidases and of HIV-I infection. Review and structure-activity relationships' RECL. TRAV. CHIM. PAYS-BAS (1993), 112(2), 82-94 , XP000979525

D6: VAN DEN BROEK, L. A. G. M. ET AL: 'Synthesis of oxygen-substituted N- alkyl

1-deoxynojirimycin derivatives: aza sugar.alpha.-glucosidase inhibitors showing antiviral (HIV-1) and immunosuppressive activity' RECL. TRAV. CHIM. PAYS-BAS (1994), 113(11), 507-16 , XP000979526

Document D1 (see claims 1 and 19) refers to the use of N-substituted-1,5-dideoxy- 1,5-imino-D-glucitol in combination therapy for treating hepatitis virus infections. Preferred compounds (see page 11, line 15 to page 14, line 14) are N-alkyl (C_6-C_{12}) substituted containing 1-5 most preferably 1-2 oxygen atoms, i.e. oxa derivates.
Document D2 (see claims) discloses the use of 1,5-(alkylimino)-1,5-dideoxy-D-glucitol in which said alkyl contains from 5 to 10 carbon atoms for inhibiting retrovirus in mammal, which presents improved spectrum of glycosidase enzyme inhibitory activity and in-vivo half life.
Document D3 (see claims and example 14 and 61) discloses a method of inhibiting lentivirus with an O-acylated derivative of 1,5-dideoxy-1,5-imino-D-glucitol.
Document D4 (see claims 1 and 12) refers to the use of N-substituted-1,5-dideoxy-1,5-imino D-glucitol or galactitol compounds for preventing, reducing or reversing multidrug resistance.
Document D5 refers to a study of chemical modification of azasugars, inhibitors of the α-glucosidase and of HIV-I infection. By alkylating the amine of 1-deoxynojirimycin (see page 84 left column to page 85 right column) the α-glucosidase inhibitory activity can be improved (N-methyl-dNM or N-butyl-dNM). However, longer alkyl chains do not show further increases in activity, e.g. N-decyl-dNM.
Document D6 (see abstract) refers to synthesis of oxygen-substituted N-alkyl 1-deoxynojirimycin derivatives. The incorporation of an oxygen atom, particularly at position seven in the N-decyl side-chain to give N-(7-oxydecyl)-dNM, the therapeutic ratio (α-glucosidase I inhibitory activity) increases considerably.

The subject-matter of claims 1-2, 5-6, 10-13,17-22 and 26-37 lacks novelty vis a vis D1 within the meaning of Article 33 (2) PCT, since the use of N-substituted-1,5-dideoxy-1,5,-imino-D-glucitol compounds for the treatment of virus infections, e.g. hepatitis is known in the art.

The problem to be solved is to provide a method of inhibiting the morphogenesis of a pestivirus or a flavivirus. The posed solution is the use of a virus inhibiting N-alkylated piperidine compound including an N-C_8-C_{16} more preferably an N-C_8-C_{10} alkyl group or an

oxa-substituted derivative thereof.

Document D1 discloses the use of N-substituted-1,5-dideoxy-1,5-imino-D-glucitol compounds for the treatment of hepatitis infection in mammal. From D6 the use of oxy substituted N-alkyl 1-deoxynojirimycin derivatives, exhibiting antiviral activity is known. Therefore, the use of a virus inhibiting N-alkylated piperidine compound including an N-C_8-C_{16} more preferably an N-C_8-C_{10} alkyl group or an oxa-substituted derivatives thereof is suggested in the state of the art and consequently the subject-matter of claims 1-6,10-13 and 17-37 does not involve an inventive step, as required by Article 33 (3) PCT.

For the assessment of the present claims 1-31 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VIII:

Figure 1 discloses N-nonyl-altrostatin. This definition seems to be not usual in the art. The applicant is requested to explain the difference between N-nonyl-DGJ and N-nonyl-altrostatin, which appear to have the same chemical structure.

The wording of claim 11 the nitrogen-containing virus inhibiting compound does not inhibit α-glucosidase and ceramide glucosyl transferase as well as N-nonyl-DNJ, leads to a lack of clarity (Article 6 PCT), since a skilled person cannot know if a virus inhibiting compound inhibits α-glucosidase and ceramide glucosyl transferase more or less than N-nonyl-DNJ.

Claim 9 seems to be superfluous, since it is identical to claim 7

The dependency of claim 16 to claim 14 is wrong. It is presumed that the applicant meant the method of claim 12.

Replaced by Article 34

No. 60/148,101 filed August 10, 1999 and U.S. Appln. No. 60/198,621 filed April 20, 2000.

From the foregoing, it would be apparent to persons skilled in the art that the invention can be embodied in other specific forms without departing from its spirit or essential characteristics. For example, all combinations of the embodiments described above are considered part of the invention with the proviso that the prior art is excluded. The described embodiments should be considered only as illustrative, not restrictive, because the scope of the invention will be indicated by the appended claims rather than by the foregoing description. All modifications which come within the meaning and range of the lawful equivalency of the claims are to be embraced within their scope. In that sense, no particular order of process steps is intended unless explicitly recited.

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* LEJA 067 PCT

Box No. I TITLE OF INVENTION

LONG CHAIN N-ALKYL COMPOUNDS AND OXA-DERIVATIVES THEREOF

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

ZITZMANN, Nicole
The Rodney Porter Building, South Parks Road
Oxford OX1 3QU, United Kingdom

☒ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State *(that is, country)* of nationality: DE

State *(that is, country)* of residence: GB

This person is applicant for the purposes of: ☒ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

BUTTERS, Terry D.
1 Pine Close, Garsington
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This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: GB

State *(that is, country)* of residence: GB

This person is applicant for the purposes of: ☒ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

KOKULIS, Paul N.
PILLSBURY MADISON & SUTRO, LLP
1100 New York Avenue, NW
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United States of America

Telephone No. 202 861-3000

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Teleprinter No.

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet is not to be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

PLATT, Frances M.
33 Millwood End, Long Hanborough
Oxfordshire OX8 8BN, United Kingdom

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: GB

State *(that is, country)* of residence: GB

This person is applicant for the purposes of: ☒ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

---

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

CARROUEE, Sandra
14 Avenue Saint Michel du Pigonnet
13090 Aix en Provence, France

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: FR

State *(that is, country)* of residence: FR

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

---

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

JACOB, Gary S.
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This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

---

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

PICKER, Donald H.
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Warren, New Jersey 07059
United States of America

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

---

☒ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Continuation of Box N . III FURTHER APPLICANTS AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet is not to be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*
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Oxfordshire OX2 7NB, United Kingdom

This person is:
☐ applicant only
☒ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: GB

State *(that is, country)* of residence: GB

This person is applicant for the purposes of: ☒ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*
DWEK, Raymond A.
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This person is:
☐ applicant only
☒ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: GB

State *(that is, country)* of residence: GB

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
☐ applicant only
☐ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
☐ applicant only
☐ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked):*

Regional Patent

☐ **AP ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, MZ Mozambique, SD Sudan, SL Sierra Leone, SZ Swaziland, TZ United Republic of Tanzania, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT

☐ **EA Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT

☒ **EP European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT

☐ **OA OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line):*

☐	**AE** United Arab Emirates	☐	**LC** Saint Lucia
☐	**AG** Antigua and Barbuda	☐	**LK** Sri Lanka
☐	**AL** Albania	☐	**LR** Liberia
☐	**AM** Armenia	☐	**LS** Lesotho
☐	**AT** Austria	☐	**LT** Lithuania
☐	**AU** Australia	☐	**LU** Luxembourg
☐	**AZ** Azerbaijan	☐	**LV** Latvia
☐	**BA** Bosnia and Herzegovina	☐	**MA** Morocco
☐	**BB** Barbados	☐	**MD** Republic of Moldova
☐	**BG** Bulgaria	☐	**MG** Madagascar
☐	**BR** Brazil	☐	**MK** The former Yugoslav Republic of Macedonia
☐	**BY** Belarus	☐	**MN** Mongolia
☐	**BZ** Belize	☐	**MW** Malawi
☐	**CA** Canada	☐	**MX** Mexico
☐	**CH and LI** Switzerland and Liechtenstein	☐	**MZ** Mozambique
☐	**CN** China	☐	**NO** Norway
☐	**CR** Costa Rica	☐	**NZ** New Zealand
☐	**CU** Cuba	☐	**PL** Poland
☐	**CZ** Czech Republic	☐	**PT** Portugal
☐	**DE** Germany	☐	**RO** Romania
☐	**DK** Denmark	☐	**RU** Russian Federation
☐	**DM** Dominica	☐	**SD** Sudan
☐	**DZ** Algeria	☐	**SE** Sweden
☐	**EE** Estonia	☐	**SG** Singapore
☐	**ES** Spain	☐	**SI** Slovenia
☐	**FI** Finland	☐	**SK** Slovakia
☐	**GB** United Kingdom	☐	**SL** Sierra Leone
☐	**GD** Grenada	☐	**TJ** Tajikistan
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☐	**KR** Republic of Korea		
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Check-boxes reserved for designating States which have become party to the PCT after issuance of this sheet:

☐ .....

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation (including fees) must reach the receiving Office within the 15-month time limit.)*

Supplemental Box *If the Supplemental Box is not used, this sheet need not be included in the request.*

1. If, in any of the Boxes, the space is insufficient to furnish all the information: in such case, write "Continuation of Box No. ..." [indicate the number of the Box] and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient, in particular:

(i) if more than two persons are involved as applicants and/or inventors and no "continuation sheet" is available: in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below;

(ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States indicated in the Supplemental Box" is checked: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;

(iii) if, in Box No. II or in any of the sub-boxes of Box No. III, the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;

(iv) if, in addition to the agent(s) indicated in Box IV, there are further agents: in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV:

(v) if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication "patent of addition," or "certificate of addition," or if, in Box No. V., the name of the United States of America is accompanied by an indication "continuation" or "continuation-in-part": in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;

(vi) if, in Box No. VI, there are more than three earlier applications whose priority is claimed: in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI;

(vii) if, in Box No. VI, the earlier application is an ARIPO application: in such case, write "Continuation of Box No. VI", specify the number of the item corresponding to that earlier application and indicate at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed.

2. If, with regard to the precautionary designation statement contained in Box No. V, the applicant wishes to exclude any State(s) from the scope of that statement: in such case, write "Designation(s) excluded from precautionary designation statement" and indicate the name or two-letter code of each State so excluded.

3. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning non-prejudicial disclosures or exceptions to lack of novelty: in such case, write "Statement concerning non-prejudical disclosures or exceptions to lack of novelty" and furnish that statement below.

Box No. IV. Agent or Common Representative: (continued)

LIPPITT, Raymond F.	BIRD, Donald J.	EDGELL, G. Paul
KNIGHT, G. Lloyd	ECCLESTON, Lynn E.	JAKOPIN, David A.
PERRY, Glenn J.	GOWDEY, Peter W.	PAULSON, Mark G.
MORDUCH, Ruth N.	LAZAR, Dale S.	KLIMA, Timothy J.
WHITE, Paul E., Jr.	McQUADE, Paul F.	COLTON, Kendrew H.
BARUFKA, Jack S.	JOYCE, Kevin E.	DZWONCZYK, Michael R.
SMYRSKI, Steven W.	SIRILLA, George M.	WISE, Roger R.
GLAZIER, Stephen C.	ZAITLEN, Richard H.	BENGTSSON, W. Patrick
NAGY, Paul G.	HESS, Adam R.	FINKELSTEIN, Jay M.
	ATKINS, William P.	SHARER, Paul L.

All attorneys are partners of the firm of PILLSBURY MADISON & SUTRO, LLP. The address, telephone number and facsimile number of all of the above attorneys are as indicated in Box IV.

Box No. VI PRIORITY CLAIM ☐ Further priority claims are indicated in the Supplemental Box.

Filing date of earlier application *(day/month/year)*	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) 10 August 1999 (10.08.99)	60/148,101	US		
item (2) 20 April 2000 (20.04.00)	60/198,621	US		
item (3)				

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): (1) & (2)

** Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):*

ISA/ EP

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority):*

Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII CHECK LIST: LANGUAGE OF FILING

This international application contains the following **number of sheets:**

request	:	6
description (excluding sequence listing part)	:	32
claims	:	6
abstract	:	1
drawings	:	8
sequence listing part of description	:	
Total number of sheets	:	53

This international application is **accompanied** by the item(s) marked below:

1. ☒ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☐ nucleotide and/or amino acid sequence listing in computer readable form
9. ☒ other *(specify):* TRANSMITTAL LETTER & POSTCARD

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: English

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Paul N. Kokulis

For receiving Office use only

1. Date of actual receipt of the purported international application:
2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority (if two or more are competent): ISA/
6. ☐ Transmittal of search copy delayed until search fee is paid.

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Date of receipt of the record copy by the International Bureau:

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 February 2001 (15.02.2001)

PCT

(10) International Publication Number
WO 01/10429 A3

(51) International Patent Classification[7]: A61K 31/435, 31/44, 31/445, 31/40, 31/195, 31/13, A61P 31/12

(21) International Application Number: PCT/US00/21732

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(26) Publication Language: English

(30) Priority Data:

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60/198,621	20 April 2000 (20.04.2000)	US

(71) Applicants and
(72) Inventors: **ZITZMANN, Nicole** [DE/GB]; The Rodney Porter Building, South Parks Road, Oxford OX1 3QU (GB). **BUTTERS, Terry, D.** [GB/GB]; 1 Pine Close, Garsington, Oxfordshire OX44 4BS (GB). **PLATT, Frances, M.** [GB/GB]; 33 Millwood End, Long Hanborough, Oxfordshire OX8 8BN (GB). **CARROUEE, Sandra** [FR/FR]; 14, avenue Saint Michel du Pigonnet, F-13090 Aix en Provence (FR). **JACOB, Gary, S.** [US/US]; 12541 Mason Forest Drive, Creve Coeur, MO 63141 (US). **PICKER, Donald, H.** [US/US]; 20 Broadway Road, Warren, NJ 07059 (US). **FLEET, George, W., J.** [GB/GB]; 187 Woodstock Road, Oxford, Oxfordshire OX2 7NB (GB). **DWEK, Raymond, A.** [GB/GB]; Ambleside, Vernon Avenue, Oxford, Oxfordshire OX2 9AU (GB).

(74) Agents: **KOKULIS, Paul, N.** et al.; Pillsbury Madison & Sutro, LLP, 1100 New York Avenue, N.W., Washington, DC 20005 (US).

(81) Designated States *(national)*: AU, BR, CA, CN, IN, JP, KR, US.

(84) Designated States *(regional)*: European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published:
— *with international search report*

(88) Date of publication of the international search report:
16 August 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/10429 A3

(54) Title: LONG CHAIN N-ALKYL COMPOUNDS AND OXA-DERIVATIVES THEREOF AND USE AS ANTIVIRAL COMPOSITIONS

(57) Abstract: Long chain N-alkyl amino and imino compounds, oxa-substituted derivatives thereof, and pharmaceutical compositions including such compounds are described. The long chain N-alkyl group is a C_8-C_{16} alkyl group. The long chain N-alkyl compounds and oxa-substituted derivatives thereof can be used in the treatment of viral infections, in particular hepatitis B virus or hepatitis C virus, in a cell or an individual. For example, the long chain N-alkyl compounds or oxa-substituted derivatives thereof can be derived from piperidines, pyrrolidines, phenylamines, pyridines, pyrroles, or amino acids.

INTERNATIONAL SEARCH REPORT

Inte. ational Application No
PCT/US 00/21732

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/435 A61K31/44 A61K31/445 A61K31/40 A61K31/195
A61K31/13 A61P31/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
CHEM ABS Data, EPO-Internal, WPI Data, PAJ, SCISEARCH, EMBASE, MEDLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 35685 A (DWEK RAYMOND A ;SEARLE & CO (US); BLOCK TIMOTHY M (US); JACOB GARY) 20 August 1998 (1998-08-20)	35
A	page 12, line 8 - line 30	3,23-25, 33-35

X	EP 0 445 098 A (SEARLE & CO) 4 September 1991 (1991-09-04)	35
A	example 4	3,23-25, 33-35
	page 2, line 31 -page 3, line 18	

X	US 5 310 745 A (PARTIS RICHARD A ET AL) 10 May 1994 (1994-05-10)	35
A	column 4, line 33	3,23-25, 33-35
	column 1, paragraph 3	

	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"P" document published prior to the international filing date but later than the priority date claimed

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 February 2001	28/02/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Bonzano, C

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/21732

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 24401 A (SEARLE & CO ;JACOB GARY S (US)) 20 May 1999 (1999-05-20)	33
A	claims 1,12	3,23-25, 33-35
A	VAN DEN BROEK, L. A. G. M. ET AL: "Chemical modification of aza sugars, inhibitors of N-glycoprotein- processing glycosidases and of HIV-I infection. Review and structure-activity relationships" RECL. TRAV. CHIM. PAYS-BAS (1993), 112(2), 82-94 , XP000979525 figure 6 table 3 page 90, column 2, paragraph 2 page 91, column 2, paragraph 3 - paragraph 7	3,23-25, 33-35
A	VAN DEN BROEK, L. A. G. M. ET AL: "Synthesis of oxygen-substituted N-alkyl 1-deoxynojirimycin derivatives: aza sugar.alpha.-glucosidase inhibitors showing antiviral (HIV-1) and immunosuppressive activity" RECL. TRAV. CHIM. PAYS-BAS (1994), 113(11), 507-16 , XP000979526 page 508, column 1 figures 2,N7	3,23-25, 33-35
A	ESPOSITO, ANNAMARIA ET AL: "Synthesis of amphiphilic polyhydroxylated pyrrolidines as potential glycosidase inhibitors" TETRAHEDRON LETT. (1998), 39(36), 6543-6546 , XP004132541 figures 5-11 page 6543, line 1 - line 11	3,23-25, 33-35
A	EP 0 367 747 A (SEARLE & CO) 9 May 1990 (1990-05-09) examples 11,12 claims 1,13 page 2, line 1 - line 25	3,23-25, 33-35

4

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1,2,4-22,26-32,36,37

Present claims 1,2,4-11,26-31,36,37 relate to compounds which are actually not well defined, since the definition specifies only part of their chemical structure: N-C8-C16 alkyl group or oxa-substituted derivative thereof.
The use of these parameters in the present context is considered to lead to a lack of clarity within the meaning of Article 6 PCT. It is impossible to compare the parameters the applicant has chosen to employ with what is set out in the prior art.
Present claims 12-22,26-32 relate to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search is impossible.
The lack of clarity is such as to render a meaningful complete search impossible. Consequently, the search has been restricted to the compounds described in claims 3,23-25,33-35.

Claims searched completely: 3,23-25,33-35.
Claims not searched: 1,2,4-22,26-32,36,37.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/21732

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9835685 A	20-08-1998	AU 6169298 A	08-09-1998
		CN 1251993 T	03-05-2000
		EP 1007058 A	14-06-2000
EP 0445098 A	04-09-1991	CA 2036964 A	27-08-1991
		JP 5085935 A	06-04-1993
		US 5030638 A	09-07-1991
US 5310745 A	10-05-1994	US 5003072 A	26-03-1991
		US 5411970 A	02-05-1995
		US 5476859 A	19-12-1995
		AT 117678 T	15-02-1995
		CA 2002106 A,C	03-05-1990
		DE 68920819 D	09-03-1995
		EP 0367748 A	09-05-1990
		ES 2070929 T	16-06-1995
		GR 3015931 T	31-07-1995
		JP 2172975 A	04-07-1990
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		US 5221746 A	22-06-1993
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		EP 1030839 A	30-08-2000
EP 0367747 A	09-05-1990	US 4876268 A	24-10-1989
		US 4937357 A	26-06-1990
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		AT 135346 T	15-03-1996
		CA 2002105 A,C	03-05-1990
		DE 68925940 D	18-04-1996
		DE 68925940 T	24-10-1996
		ES 2086323 T	01-07-1996
		GR 3019885 T	31-08-1996
		JP 2172972 A	04-07-1990
		JP 2840334 B	24-12-1998

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(43) International Publication Date
15 February 2001 (15.02.2001)

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(21) International Application Number: PCT/US00/21732

(22) International Filing Date: 10 August 2000 (10.08.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/148,101	10 August 1999 (10.08.1999)	US
60/198,621	20 April 2000 (20.04.2000)	US

(71) Applicants and
(72) Inventors: **ZITZMANN, Nicole** [DE/GB]; The Rodney Porter Building, South Parks Road, Oxford OX1 3QU (GB). **BUTTERS, Terry, D.** [GB/GB]; 1 Pine Close, Garsington, Oxfordshire OX44 4BS (GB). **PLATT, Frances, M.** [GB/GB]; 33 Millwood End, Long Hanborough, Oxfordshire OX8 8BN (GB). **CARROUEE, Sandra** [FR/FR]; 14, avenue Saint Michel du Pigonnet, F-13090 Aix en Provence (FR). **JACOB, Gary, S.** [US/US]; 12541 Mason Forest Drive, Creve Coeur, MO 63141 (US). **PICKER, Donald, H.** [US/US]; 20 Broadway Road, Warren, NJ 07059 (US). **FLEET, George, W., J.** [GB/GB]; 187 Woodstock Road, Oxford, Oxfordshire OX2 7NB (GB). **DWEK, Raymond, A.** [GB/GB]; Ambleside, Vernon Avenue, Oxford, Oxfordshire OX2 9AU (GB).

(74) Agents: **KOKULIS, Paul, N.** et al.; Pillsbury Madison & Sutro, LLP, 1100 New York Avenue, N.W., Washington, DC 20005 (US).

(81) Designated States *(national)*: AU, BR, CA, CN, IN, JP, KR, US.

(84) Designated States *(regional)*: European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published:
— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/10429 A2

(54) Title: LONG CHAIN N-ALKYL COMPOUNDS AND OXA-DERIVATIVES THEREOF

(57) Abstract: Long chain N-alkyl amino and imino compounds, oxa-substituted derivatives thereof, and pharmaceutical compositions including such compounds are described. The long chain N-alkyl group is a C_8-C_{16} alkyl group. The long chain N-alkyl compounds and oxa-substituted derivatives thereof can be used in the treatment of viral infections, in particular hepatitis B virus or hepatitis C virus, in a cell or an individual. For example, the long chain N-alkyl compounds or oxa-substituted derivatives thereof can be derived from piperidines, pyrrolidines, phenylamines, pyridines, pyrroles, or amino acids.

LONG CHAIN N-ALKYL COMPOUNDS AND OXA-DERIVATIVES THEREOF

FIELD OF THE INVENTION

This invention relates to long chain N-alkyl amino and imino compounds and oxa-derivatives thereof for treating pestivirus and flavivirus infections of animals and humans.

BACKGROUND OF THE INVENTION

HCV is an RNA virus belonging to the *Flaviviridae* family. Individual isolates consist of closely related, yet heterologous populations of viral genomes. This genetic diversity enables the virus to escape the host's immune system, leading to a high rate of chronic infection. The flavivirus group to which HCV belongs is known to include the causative agents of numerous human diseases transmitted by arthropod vectors. Human diseases caused by flaviviruses include various hemorrhagic fevers, hepatitis, and encephalitis. Viruses known to cause these diseases in humans have been identified and include, for example, yellow fever virus, dengue viruses 1-4, Japanese encephalitis virus, Murray Valley encephalitis virus, Rocio virus, West Nile fever virus, St. Louis encephalitis virus, tick-borne encephalitis virus, Louping ill virus, Powassan virus, Omsk hemorrhagic fever virus, and Kyasanur forest disease virus. A critical need therefore also exists for treating animals, as well as humans, infected with at least one virus, such as a flavivirus and/or pestivirus.

More than 40 million people worldwide are chronically infected with the hepatitis C virus (HCV), and this represents one of the most serious threats to the public health of developed nations (Hoofnagle et al., *New Engl. J. Med.* 336:347-356, 1997). Hepatitis C infection is the cause of more than 10,000 deaths annually in the United States (*Washington Post*, November 11, 1997, at A2), a number that is expected to triple in the next twenty years in the absence of effective intervention. Chronic HCV also increases the risk of liver cancer. There are more than 40 million people worldwide who are chronically infected with HCV, representing one of the most serious threats to the public health of developed nations (Hoofnagle et al., *ibid.*). Persistent infection develops in as many as 85% of HCV patients and in at least 20% of these patients the chronic infection leads to cirrhosis within twenty years of onset of infection. With an estimated 3.9 million North Americans chronically infected, complications from hepatitis C infection are now the leading reasons for liver transplantation in the United States.

Another causative agent of acute and chronic liver disease including liver fibrosis, cirrhosis, inflammatory liver disease, and hepatic cancer is hepatitis B virus (HBV) (Joklik, *Virology,* 3rd Ed., Appleton & Lange, Norwalk, Connecticut, 1988). Although effective vaccines are available, there are still more than 300 million people worldwide, i.e., 5% of the world's population, chronically infected with the virus (Locamini et al., *Antiviral Chemistry & Chemotherapy* 7:53-64, 1996). Such vaccines have no therapeutic value for those already infected with the virus. In Europe and North America, between 0.1% to 1% of the population is infected. Estimates are that 15% to 20% of individuals who acquire the infection develop cirrhosis or another chronic disability from HBV infection. Once liver cirrhosis is established, morbidity and mortality are substantial, with about a 5-year patient survival period (Blume et al., *Advanced Drug Delivery Reviews* 17:321-331, 1995). It is therefore necessary and of high priority to find improved and effective anti-HBV anti-hepatitis therapies (Locamini et al., *ibid.*).

Therapeutic interventions which are effective for treatment of HCV infection are limited in number and effectiveness. Standard treatment for HCV infection includes administration of interferon-alpha. However, interferon-alpha is of limited use in about 20% of the HCV-infected population (Hoofnagle et al., *ibid.*) and treatment with this compound results in long-term improvement in only 5% of patients. Furthermore, the complications and limitations of interferon-alpha seriously limit the applicability of the treatment. An experimental treatment comprising administration of interferon-alpha and ribavirin (1-β-*D*-ribofuranosyl-1H-1,2,4-triazole-3-carboxamide) resulted in long-term improvement in only half of the patients suffering a relapse of HCV infection (*Washington Post*, November 11, 1997, at A2). Clearly, the disappointing results with interferon must prompt a search for more effective and less toxic therapeutics. Thus, a critical need remains for a therapeutic intervention that effectively treats HCV infection or supplements those otherwise available.

In addition to those people chronically infected with HCV, there are more than 350 million people chronically infected with hepatitis B virus (HBV). More than 150 million of these people are likely to die from liver disease in the absence of intervention. As many as 20 million HBV carriers reside in developed nations, as do most HCV carriers. A large number of individuals who are infected with HCV are also infected with HBV. The therapy for combined HBV/HCV infection is particularly challenging because the HBV and HCV viruses differ from one another in therapeutically significant ways. HBV is a hepadnavirus, while HCV is a pestivirus. HBV is a DNA-containing virus, the genome of which is

replicated in the nucleus of the infected cell using a combination of a DNA-dependent RNA polymerase and an RNA-dependent DNA polymerase (i.e., a reverse transcriptase). HCV is an RNA-containing virus, the genome of which is replicated in the cytoplasm of the infected cell using one or more types of RNA-dependent RNA polymerases. Despite the frequent concurrence of HBV infection and HCV infection, a number of compounds known to be effective for treating HBV infection are not effective against HCV. For example, lamivudine (the nucleoside analog 3TC) is useful for treating HBV infection, but is not useful for treating HCV infection. The difference in the susceptibility of HBV and HCV to antiviral agents no doubt relates to their genetically based replicative differences. There remains a particularly critical need for a therapeutic intervention that effectively treats both HBV and HCV infection.

Other hepatitis viruses significant as agents of human disease include hepatitis A, hepatitis Delta, hepatitis E, hepatitis F, and hepatitis G (Coates et al., *Exp. Opin. Ther. Patents* 5:747-756, 1995). In addition, there are animal hepatitis viruses that are species specific. These include, for example, those infecting ducks, woodchucks, and mice. The availability of animal models allows the preclinical testing of antiviral compounds for each class of virus. Furthermore, animal viruses can cause significant losses to the livestock industry (Sullivan et al., *Virus Res.* 38:231-239, 1995). Such animal viruses include pestiviruses and flaviviruses such as bovine viral diarrhea virus (BVDV), classical swine fever virus, border disease virus, and hog cholera virus.

SUMMARY OF THE INVENTION

In general, the invention features long chain N-alkyl amino and imino compounds and oxa-substituted derivatives thereof and includes pharmaceutical compositions containing an effective amount of such compounds. The long chain N-alkyl group is a C_8-C_{16} alkyl group. The long chain N-alkyl compounds and oxa-substituted derivatives thereof can be used in the treatment of viral infections in a cell or an individual. In an individual, the infection may result in chronic or acute disease and treatment of same may reduce the severity of infection (e.g., production of virus) or disease symptoms. The long chain N-alkyl compounds may or may not inhibit glycosidase activity or glycoplipid synthesis at a detectable level; preferred are compounds that do not inhibit α-glucosidase activity at a detectable level but still are effective in treating infection. For example, the long chain N-alkyl compounds and oxa-substituted derivatives can be derived from a piperidine, a pyrrolidine, a phenylamine, a

pyridine, a pyrrole, or an amino acid.

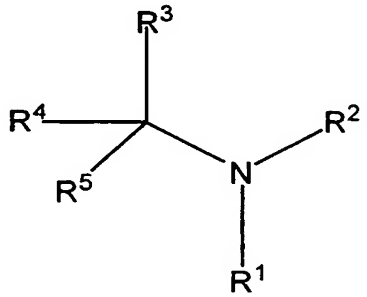
In one aspect, the invention features a nitrogen-containing virus-inhibiting compound including an N-C_8-C_{16} alkyl group. Preferably, the compound includes an N-C_8-C_{10} alkyl group (e.g., N-nonyl or N-decyl group) or an N-C_8-C_{10} oxa-alkyl group such as an N-$(CH_2)_6O(CH_2)_nCH_3$ group or N-$(CH_2)_2O(CH_2)_{n+4}CH_3$ group for n = 1, 2 or 3. The nitrogen-containing virus-inhibiting compound can have an inhibitory concentration (IC_{50}) of about 20 μM or less, preferably about 10 μM or less, and more preferably about 5 μM or less, for the inhibition of one or more pestiviruses or a flaviviruses in an assay (e.g., plaque formation, yield). In particular, a compound effective against both a pestivirus and a flavivirus (e.g., HBV and BVDV) is preferred.

In another aspect, the invention features a method of inhibiting morphogenesis of a virus. The method includes administering an effective amount of the nitrogen-containing virus-inhibiting compound, or a pharmaceutically acceptable salt thereof, to a cell or an individual infected with the virus. The cell can be a mammalian cell or a human cell.

In yet another aspect, the invention features a method of treating an individual infected with a virus. The method includes administering an effective amount of the nitrogen-containing virus-inhibiting compound, or a pharmaceutically acceptable salt thereof, to an individual infected with a virus. The treatment can reduce, abate, or diminish the virus infection in the animal or human. The animal can be a bird or mammal (e.g., pig, cow, mice). The nitrogen-containing virus-inhibiting compound can be administered orally.

In another aspect, the invention features a method of manufacturing a pharmaceutical composition comprising combining at least one nitrogen-containing virus-inhibiting compound including an N-C_8-C_{16} alkyl group or an oxa-substituted derivative thereof with a pharmaceutically acceptable carrier.

The compound can have the formula:



in which R^1 is a C_8-C_{16} alkyl; and can also contain 1 to 5, preferably 1 to 3, and more preferably 1 to 2 oxygen atoms (i.e., oxa-substituted derivatives). Preferred oxa-substituted

derivatives are 3-oxanonyl, 3-oxadecyl, 7-oxanonyl and 7-oxadecyl.

R^2 is hydrogen, R^3 is carboxy, or a C_1-C_4 alkoxycarbonyl, or R^2 and R^3, together are

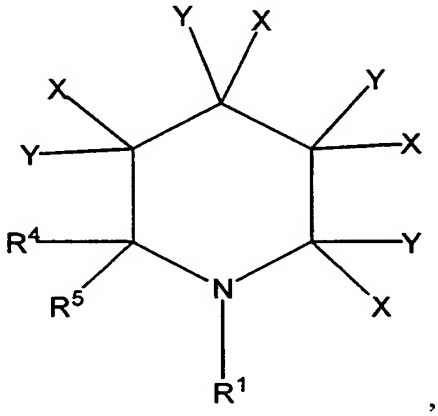
$-(\ C\ )_n-$ or $-(CXY)_n-$, wherein n is 3 or 4, each X, independently, is hydrogen, hydroxy, amino, carboxy, a C_1-C_4 alkylcarboxy, a C_1-C_4 alkyl, a C_1-C_4 alkoxy, a C_1-C_4 hydroxyalkyl, a C_1-C_6 acyloxy, or an aroyloxy, and each Y, independently, is hydrogen, hydroxy, amino, carboxy, a C_1-C_4 alkylcarboxy, a C_1-C_4 alkyl, a C_1-C_4 alkoxy, a C_1-C_4 hydroxyalkyl, a C_1-C_6 acyloxy, an aroyloxy, or deleted (i.e., not present);

R^4 is hydrogen or deleted (i.e., not present); and

R^5 is hydrogen, hydroxy, amino, a substituted amino, carboxy, an alkoxycarbonyl, an aminocarbonyl, an alkyl, an aryl, an aralkyl, an alkoxy, a hydroxyalkyl, an acyloxy, or an aroyloxy, or R^3 and R^5, together, form a phenyl and R^4 is deleted (i.e., not present). When R^2 and R^3, together, are $-(CXY)_n-$ and R^4 is deleted (i.e., not present), all Y are deleted (i.e., not present). The compound can be a physiologically acceptable salt or solvate of the compound.

In certain embodiments, R^1 is a C_8-C_{10} alkyl (e.g., C_9 alkyl) and R^2 can be hydrogen, R^3 can be carboxy, or a C_1-C_4 alkoxycarbonyl, R^4 can be hydrogen, and R^5 can be hydrogen, hydroxy, amino, a substituted amino, carboxy, an alkoxycarbonyl, an aminocarbonyl, an alkyl, an aryl, an aralkyl, an alkoxy, a hydroxyalkyl, an acyloxy, or an aroyloxy. In certain preferred embodiments, R^3 is carboxy. In other preferred embodiments, R^3 and R^5, together, form a phenyl and R^4 is deleted (i.e., not present). In yet other preferred embodiments, R^2 and R^3, together, are $-(CXY)_n-$.

In certain embodiments, the compound has the formula:



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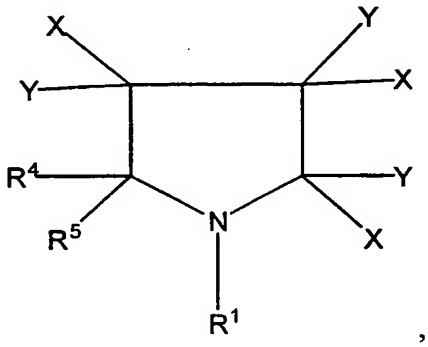

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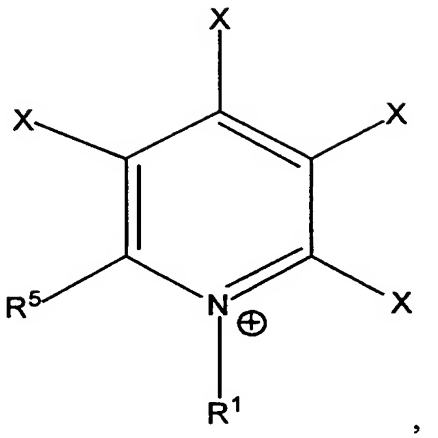

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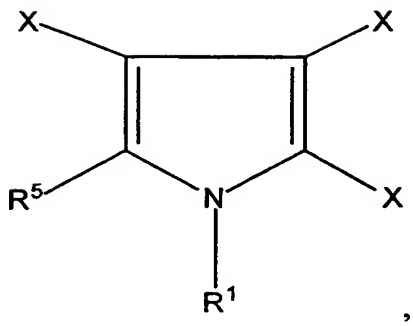

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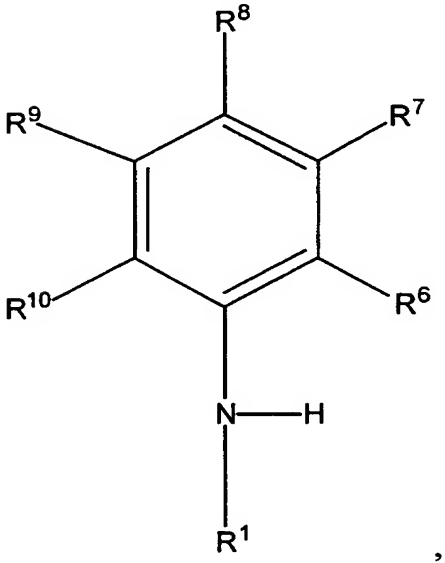
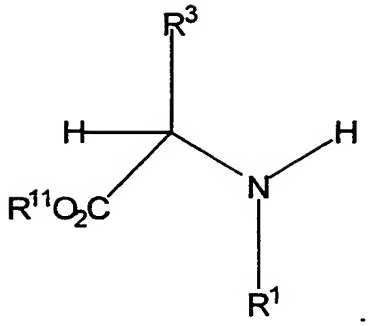




, or .

Each of R^6-R^{10}, independently, is hydrogen, hydroxy, amino, carboxy, a C_1-C_4 alkylcarboxy, a C_1-C_4 alkyl, a C_1-C_4 alkoxy, a C_1-C_4 hydroxyalkyl, a C_1-C_6 acyloxy, or an aroyloxy, and R^{11} is hydrogen, or a C_1-C_4 alkyl.

The nitrogen-containing virus inhibiting compound can be N-alkylated piperidines, N-oxa-alkylated piperidines, N-alkylated pyrrolidines, N-oxa-alkylated pyrrolidines, N-alkylated phenylamines, N-oxa-alkylated phenylamines, N-alkylated pyridines, N-oxa-alkylated pyridines, N-alkylated pyrroles, N-oxa-alkylated pyrroles, N-alkylated amino acids, or N-oxa-alkylated amino acids. In certain embodiments, the N-alkylated piperidine, N-oxa-alkylated piperidine, N-alkylated pyrrolidine, or N-oxa-alkylated pyrrolidine compound can be an imino sugar. For example, preferred nitrogen-containing virus-inhibiting compounds are N-nonyl-1,5-dideoxy-1,5-imino-D-galactitol (N-nonyl-deoxygalactonojirimycin or N-nonyl DGJ), N-(7-oxa-nonyl)-1,5-dideoxy-1,5-imino-D-galactitol (N-7-oxa-nonyl DGJ), N-nonyl-1,5,6-trideoxy-1,5-imino-D-galactitol (N-nonyl MeDGJ), N-(7-oxa-nonyl)-1,5,6-

trideoxy-1,5-imino-D-galactitol (N-7-oxa-nonyl MeDGJ), N-nonyl altrostatin, N-nonyl-*2R,5R*-dihydroxymethyl-*3R,4R*-dihydroxypyrrolidine (N-nonyl DMDP), N-nonyl-deoxynojirimycin (N-nonyl DNJ), N-nonyl-2-aminobenzamide (2ABC9), or a derivative, an enantiomer or a stereoisomer thereof. The structures of unsubstituted compounds are shown in Figure 1.

In certain embodiments, the virus can be a flavivirus or a pestivirus. Infections by flaviviruses include, but are not limited to, those caused by a yellow fever virus, a dengue virus (e.g., dengue viruses 1-4), a Japanese encephalitis virus, a Murray Valley encephalitis virus, a Rocio virus, a West Nile fever virus, a St. Louis encephalitis virus, a tick-borne encephalitis virus, a Louping ill virus, a Powassan virus, an Omsk hemorrhagic fever virus, and a Kyasanur forest disease virus. Infections by pestiviruses include, but are not limited to, those caused by hepatitis C virus (HCV), rubella virus, a bovine viral diarrhea virus (BVDV), a classical swine fever virus, a border disease virus, or a hog cholera virus.

According to yet another aspect, the invention features a prophylactic method for protecting a mammal infected by a virus from developing hepatitis or a heptacellular cancer that is among the sequelae of infection by the virus, including administering to the virus infected cell of the animal an effective anti-viral amount of the nitrogen-containing virus-inhibiting compound.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts chemical structures for compounds which were used in this study.

Figure 2 depicts the percent of BVDV plaques produced by an infected cell culture in the presence of various concentrations of compounds: N-butyl DGJ (◆), N-nonyl DGJ (■), N-nonyl MeDGJ (▲), or N-nonyl DNJ(×).

Figures 3 depicts the IC_{50} of various alkyl lengths of N-alkylated compounds and Figure 5 depicts the IC_{50} of N-nonyl compounds.

Figure 4 depicts the percent of BVDV plaques produced by an infected cell culture in the presence of various concentrations of N-nonyl DGJ (▲) or N-decyl DGJ (×).

Figure 6 depicts the percent of BVDV plaques produced by an infected cell culture in the presence of various concentrations of N-nonyl compounds: 2ABC9 (◆), nonylamine (■), N-nonyl-altrostatin (△), N-nonyl-DGJ (×), N-nonyl-MeDGJ (✳), N-nonyl-DNJ (●), or N-nonyl-DMDP (+).

Figure 7 depicts the percent of BVDV plaques produced by an infected cell culture in

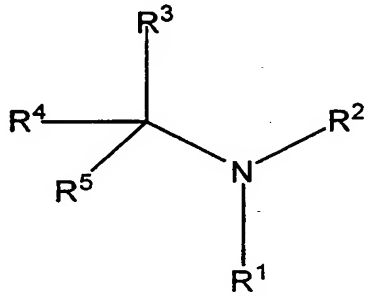
the presence of various concentrations of N-7-oxa-nonyl MeDGJ.

Figure 8 depicts the increasing uptake of ^{3}H-labeled inhibitors in HepG2 cells in the following order: N-butyl-DNJ (◆), N-hexyl-DNJ (■), N-octyl-DNJ (▲), N-nonyl-DNJ (×), N-decyl-DNJ (ж), N-dodecyl-DNJ (●), N-hexadecan-DNJ (+), or N-octadecan-DNJ (—).

DESCRIPTION OF THE INVENTION

The nitrogen-containing virus-inhibiting compound includes an N-C_8-C_{16} alkyl group, such as an N-C_8-C_{10} alkyl group, particularly a nonyl or decyl group, or an oxa-substituted derivative thereof. The nitrogen-containing virus-inhibiting compound can be an N-alkylated piperidine, N-oxa-alkylated piperidine, N-alkylated pyrrolidine, N-oxa-alkylated pyrrolidine, N-alkylated phenylamine, N-oxa-alkylated phenylamine, N-alkylated pyridine, N-oxa-alkylated pyridine, N-alkylated pyrrole, N-oxa-alkylated pyrrole, N-alkylated amino acid, or N-oxa-alkylated amino acid such as N-nonyl DGJ, N-oxa-nonyl DGJ, N-nonyl MeDGJ, N-oxa-nonyl MeDGJ, N-nonyl altrostatin, N-nonyl DMDP, N-oxa-nonyl DMDP, N-nonyl-2-aminobenzamide, or N-oxa-nonyl-2-aminobenzamide.

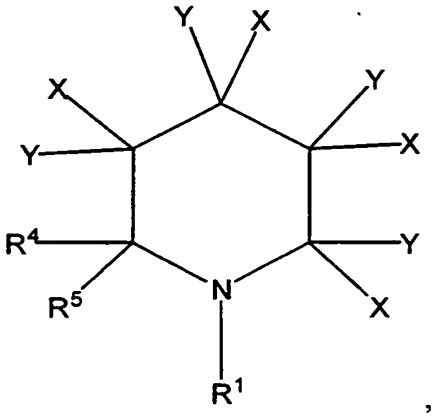
The compound can have the formula:



in which R^1 is a C_8-C_{16} alkyl, R^2 is hydrogen, R^3 is carboxy, or a C_1-C_4 alkoxycarbonyl, R^4 is hydrogen, and R^5 is hydrogen, hydroxy, amino, a substituted amino, carboxy, an alkoxycarbonyl, an aminocarbonyl, an alkyl, an aryl, an aralkyl, an alkoxy, a hydroxyalkyl, an acyloxy, or an aroyloxy. Alternatively, R^1 is a C_8-C_{16} alkyl, R^2 is hydrogen, R^3 and R^5, together, form a phenyl, which can be substituted or unsubstituted, and R^4 is deleted (i.e., not present). In another alternative, R^1 is a C_8-C_{16} alkyl, R^4 is hydrogen or deleted (i.e., not present), R^5 is hydrogen, hydroxy, amino, a substituted amino, carboxy, an alkoxycarbonyl, an aminocarbonyl, an alkyl, an aryl, an aralkyl, an alkoxy, a hydroxyalkyl, an acyloxy, or an aroyloxy, and R^2 and R^3, together, are $-(\overset{X\ Y}{\backslash\ /\ }C\ )_n-$ or $-(CXY)_n-$, wherein n is 3 or 4, each X, independently, is hydrogen, hydroxy, amino, carboxy, a C_1-C_4 alkylcarboxy, a C_1-C_4 alkyl, a

C_1-C_4 alkoxy, a C_1-C_4 hydroxyalkyl, a C_1-C_6 acyloxy, or an aroyloxy, and each Y, independently, is hydrogen, hydroxy, amino, carboxy, a C_1-C_4 alkylcarboxy, a C_1-C_4 alkyl, a C_1-C_4 alkoxy, a C_1-C_4 hydroxyalkyl, a C_1-C_6 acyloxy, an aroyloxy, or deleted. When R^2 and R^3, together, are $-(CXY)_n-$ and R^4 is deleted, all Y are deleted. The compound can be a physiologically acceptable salt or solvate of the compound.

In certain embodiments, the compound has the formula:



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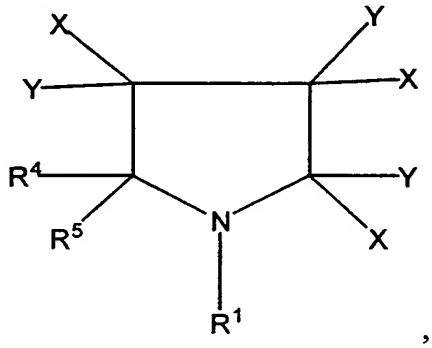

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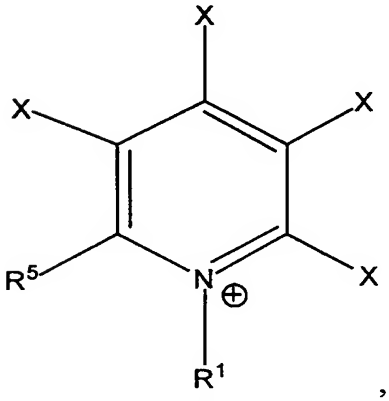

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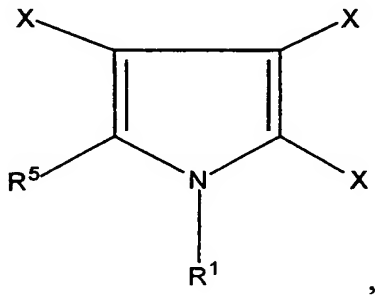

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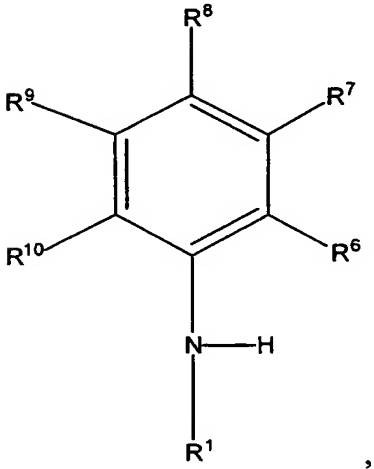

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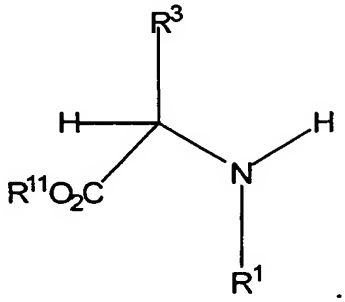

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Each of R^6-R^{10}, independently, is hydrogen, hydroxy, amino, carboxy, a C_1-C_4

alkylcarboxy, a C_1-C_4 alkyl, a C_1-C_4 alkoxy, a C_1-C_4 hydroxyalkyl, a C_1-C_6 acyloxy, or an aroyloxy, and R^{11} is hydrogen, or a C_1-C_4 alkyl.

As used herein, the groups have the following characteristics, unless the number of carbon atoms is specified otherwise. Alkyl groups have from 1 to 16 carbon atoms and are linear or branched, substituted or unsubstituted. Alkoxy groups have from 1 to 16 carbon atoms, and are linear or branched, substituted or unsubstituted. Alkoxycarbonyl groups are ester groups having from 2 to 16 carbon atoms. Alkenyloxy groups have from 2 to 16 carbon atoms, from 1 to 6 double bonds, and are linear or branched, substituted or unsubstituted. Alkynyloxy groups have from 2 to 16 carbon atoms, from 1 to 3 triple bonds, and are linear or branched, substituted or unsubstituted. Aryl groups have from 6 to 14 carbon atoms (e.g., phenyl groups) and are substituted or unsubstituted. Aralkyloxy (e.g., benzyloxy) and aroyloxy (e.g., benzoyloxy) groups have from 7 to 15 carbon atoms and are substituted or unsubstituted. Amino groups can be primary, secondary, tertiary, or quaternary amino groups (i.e., substituted amino groups). Aminocarbonyl groups are amido groups (e.g., substituted amido groups) having from 1 to 32 carbon atoms. Substituted groups can include a substituent selected from the group consisting of halogen, hydroxy, $C_{1\text{-}10}$ alkyl, $C_{2\text{-}10}$ alkenyl, $C_{1\text{-}10}$ acyl, or $C_{1\text{-}10}$ alkoxy.

The N-alkylated amino acid can be an N-alkylated naturally occurring amino acid, such as an N-alkylated α-amino acid. A naturally occurring amino acid is one of the 20 common α-amino acids (Gly, Ala, Val, Leu, Ile, Ser, Thr, Asp, Asn, Lys, Glu, Gln, Arg, His, Phe, Cys, Trp, Tyr, Met, and Pro), and other amino acids that are natural products, such as norleucine, ethylglycine, ornithine, methylbutenyl-methylthreonine, and phenylglycine. Examples of amino acid side chains (e.g., R^5) include H (glycine), methyl (alanine), $-CH_2C(O)NH_2$ (asparagine), $-CH_2$-SH (cysteine), and $-CH(OH)CH_3$ (threonine).

A long chain N-alkylated compound can be prepared by reductive alkylation of an amino (or imino) compound. For example, the amino or imino compound can be exposed to a long chain aldehyde, along with a reducing agent (e.g., sodium cyanoborohydride) to N-alkylate the amine. Similarly, a long chain N-oxa-alkylated compound can be prepared by reductive alkylation of an amino (or imino) compound. For example, the amino or imino compound can be exposed to a long chain oxa-aldehyde, along with a reducing agent (e.g., sodium cyanoborohydride) to N-oxa-alkylate the amine.

The compounds can include protecting groups. Various protecting groups are well known. In general, the species of protecting group is not critical, provided that it is stable to

the conditions of any subsequent reaction(s) on other positions of the compound and can be removed at the appropriate point without adversely affecting the remainder of the molecule. In addition, a protecting group may be substituted for another after substantive synthetic transformations are complete. Clearly, where a compound differs from a compound disclosed herein only in that one or more protecting groups of the disclosed compound has been substituted with a different protecting group, that compound is within the invention. Further examples and conditions are found in Greene, *Protective Groups in Organic Chemistry*, (1st Ed., 1981, Greene & Wuts, 2nd Ed., 1991).

The compounds can be purified, for example, by crystallization or chromatographic methods. The compound can be prepared stereospecifically using a stereospecific amino or imino compound as a starting material.

The amino and imino compounds used as starting materials in the preparation of the long chain N-alkylated compounds are commercially available (Sigma, St. Louis, MO; Cambridge Research Biochemicals, Norwich, Cheshire, United Kingdom; Toronto Research Chemicals, Ontario, Canada) or can be prepared by known synthetic methods. For example, the compounds can be long chain N-alkylated imino sugar compounds or oxa-substituted derivatives thereof. The imino sugar can be, for example, deoxygalactonojirmycin (DGJ), 1-methyl-deoxygalactonojirimycin (MeDGJ), deoxynorjirimycin (DNJ), altrostatin, 2*R*,5*R*-dihydroxymethyl-3*R*,4*R*-dihydroxypyrrolidine (DMDP), or derivatives, enantiomers, or stereoisomers thereof.

The syntheses of a variety of imino sugar compounds have been described. For example, methods of synthesizing DNJ derivatives are known and are described, for example, in U.S. Patent Nos. 5,622,972, 5,200,523, 5,043,273, 4,994,572, 4,246,345, 4,266,025, 4,405,714, and 4,806,650, and U.S. patent application 07/851,818, filed March 16, 1992. Methods of synthesizing other imino sugar derivatives are known and are described, for example, in U.S. Patent Nos. 4,861,892, 4,894,388, 4,910,310, 4,996,329, 5,011,929, 5,013,842, 5,017,704, 5,580,884, 5,286,877, and 5,100,797. The enantiospecific synthesis of 2*R*,5*R*-dihydroxymethyl-3*R*,4*R*-dihydroxypyrrolidine (DMDP) is described by Fleet & Smith (*Tetrahedron Lett.* 26:1469-1472, 1985).

The substituents on the imino sugar compound can influence the potency of the compound as an antiviral agent and additionally can preferentially target the molecule to one organ rather than another. Methods for comparing the potencies of various substituted compounds are provided in the Examples.

With the exception of the pyridinium compounds, which are in salt form, the compounds described herein may be used in the free amine form or in a pharmaceutically acceptable salt form. The counter anion of the pyridinium compound can be chloride, tartrate, phosphate, or sulfate. Pharmaceutical salts and methods for preparing salt forms are provided by Berge et al. (*J. Pharm. Sci.* 66:1-18, 1977). Pharmaceutically acceptable salts can be preferred for compounds that are difficult to solubilize in the pharmaceutical composition (e.g., compounds having longer alkyl chains). A salt form is illustrated, for example, by the HCl salt of an amino derivative. The compounds may also be used in the form of prodrugs, such as the 6-phosphorylated DNJ derivatives described in U.S. Patents Nos. 5,043,273 and 5,103,008. Use of compositions which further comprise a pharmaceutically acceptable carrier and compositions which further comprise components useful for delivering the composition to an animal are explicitly contemplated. Numerous pharmaceutically acceptable carriers useful for delivering the compositions to a human and components useful for delivering the composition to other animals such as cattle are known in the art. Addition of such carriers and components to the composition of the invention is well within the level of ordinary skill in the art. For example, the compounds can be di- or tetra- acetates, propionates, butyrates, or isobutyrates. The compound can be a solvate.

The invention also encompasses isotopically-labeled counterparts of compounds disclosed herein. An isotopically-labeled compound of the invention has one or more atoms replaced with an isotope having a detectable particle- or x-ray-emitting (radioactive) nucleus or a magnetogyric nucleus. Examples of such nuclei include ^{2}H, ^{3}H, ^{13}C, ^{15}N, ^{19}F, ^{29}Si, ^{31}P, ^{32}P and ^{125}I. Isotopically-labeled compounds of the invention are particularly useful as probes or research tools for spectrometric analyses, radioimmunoassays, binding assays based on scintillation, fluorography, autoradiography, and kinetic studies such as inhibition studies or determination of primary and secondary isotope effects.

The nitrogen-containing virus-inhibiting compound can be administered to a cell or an individual affected by a virus. The compound can inhibit morphogenesis of the virus, or it can treat the individual. The treatment can reduce, abate, or diminish the virus infection in the animal. For example, the N-nonyl, N-decyl, N-3-oxa-nonyl, N-3-oxa-decyl, N-7-oxa-nonyl, and N-7-oxa-decyl compounds are antiviral. The antiviral activity is substantially unrelated to the remaining functionalities of the compound.

The nitrogen-containing virus-inhibiting compound combined with at least one other antiviral compound, such as an inhibitor of a viral DNA or RNA polymerase and/or protease,

and/or at least one inhibitor of expression of viral genes, replication of the viral genome, and/or assembly of a viral particle. The supplemental antiviral compound may be any antiviral agent, which is presently recognized, or any antiviral agent which becomes recognized. By way of example, the supplemental antiviral compound may be interferon-alpha, interferon-beta, ribavirin, lamivudine, brefeldin A, monensin, TUVIRUMAB™ (Protein Design Labs) PENCICLOVIR™ (SmithKline Beecham), FAMCICLOVIR™ (SmithKline Beecham), BETASERON™ (Chiron), THERADIGM-HBV™ (Cytel), Adefovir Dipivoxil (GS 840, Gilead Sciences), INTRON A™ (Schering Plough), ROFERON™ (Roche Labs), BMS 200,475 (Bristol Myers Squibb), LOBUCAVIR™ (Bristol Myers Squibb), FTC (Triangle Pharmaceuticals), DAPD (Triangle Pharmaceuticals), thymosin alpha peptide, Glycovir (Block et al., *Proc. Natl. Acad. Sci. USA* 91:2235-2240, 1994), granulocyte macrophage colony stimulating factor (Martin et al., *Hepatology* 18:775-780, 1993), an "immune-cytokine" (Guidotti et al., *J. Virol.* 68:1265-1270, 1994), CDG (Fourel et al., *J. Virol.* 68:1059-1065, 1994), or the like.

Long chain N-alkyl compounds are agents that exhibit an inhibitory effect on viral expression. While certain short chain N-alkyl derivatives of imino sugars (e.g., N-butyl DNJ) are potent inhibitors of the N-linked oligosaccharide processing enzymes, such as α-glucosidase I and α-glucosidase II (Saunier et al., *J. Biol. Chem.* 257:14155-14161, 1982; Elbein, *Ann. Rev. Biochem.* 56:497-534, 1987). Some long chain N-alkyl compounds of the invention may exhibit substantially little or no inhibition of a glycosidase enzyme, especially in comparison with N-butyl DNJ or N-nonyl DNJ. Unexpectedly, some long chain N-alkyl compounds do effectively inhibit viral morphogenesis in cells infected with a virus, such as a flavivirus or pestivirus. For example, the nitrogen-containing virus-inhibiting compound can have an IC_{50} of about 10 μM or less, preferably about 3 μM or less, for the inhibition of BVDV or another virus, but the same compounds may exhibit little activity against glycosidases or inhibition of glycolipid synthesis.

Methods for treating a mammal infected with respiratory syncytial virus (RSV) using DNJ derivatives have been described in U.S. Patent No. 5,622,972. The use of DNJ and N-methyl-DNJ has also been disclosed to interrupt the replication of non-defective retroviruses such as human immunodeficiency virus (HIV), feline leukemia virus, equine infectious anemia virus, and lentiviruses of sheep and goats (U.S. Patent Nos. 5,643,888 and 5,264,356; Acosta et al., *Am. J. Hosp. Pharm.* 51:2251-2267, 1994).

In the absence of a suitable cell culture system able to support replication of human

HCV, bovine viral diarrhea virus (BVDV) serves as the FDA approved model organism for HCV, as both share a significant degree of local protein region homology (Miller & Purcell, *Proc. Natl. Acad. Sci. USA* 87:2057-2061, 1990), common replication strategies, and probably the same subcellular location for viral envelopment. Compounds found to have an antiviral effect against BVDV are highly recommended as potential candidates for treatment of HCV.

The cytotoxicity resulting from exposure of mammalian cells in tissue culture to bovine viral diarrhea virus (BVDV) is prevented by addition of a nitrogen-containing virus-inhibiting compound to the tissue culture medium. The virus inhibitors that were used in the examples below included long chain N-alkyl derivatives of DGJ. Because BVDV is an accepted tissue culture model of HCV (Henzler & Kaiser, *Nature Biotechnology* 16:1077-1078, 1998), the compositions and methods described herein for inhibiting morphogenesis of BVDV are also useful for inhibiting morphogenesis of HCV.

The amount of antiviral agent administered to an animal or to an animal cell according to the methods of the invention is an amount effective to inhibit the viral morphogenesis from the cell. The term "inhibit" as used herein refers to the detectable reduction and/or elimination of a biological activity exhibited in the absence of a nitrogen-containing virus-inhibiting compound according to the invention. The term "effective amount" refers to that amount of composition necessary to achieve the indicated effect. The term "treatment" as used herein refers to reducing or alleviating symptoms in a subject, preventing symptoms from worsening or progressing, inhibition or elimination of the causative agent, or prevention of the infection or disorder in a subject who is free therefrom.

Thus, for example, treatment of viral infection includes destruction of the infecting agent, inhibition of or interference with its growth or maturation, neutralization of its pathological effects, and the like. The amount of the composition which is administered to the cell or animal is preferably an amount that does not induce any toxic effects which outweigh the advantages which accompany its administration.

Actual dosage levels of active ingredients in the pharmaceutical compositions of this invention may be varied so as to administer an amount of the active compound(s) that is effective to achieve the desired therapeutic response for a particular patient.

The selected dose level will depend on the activity of the selected compound, the route of administration, the severity of the condition being treated, and the condition and prior medical history of the patient being treated. However, it is within the skill of the art to

start doses of the compound(s) at levels lower than required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. If desired, the effective daily dose may be divided into multiple doses for purposes of administration, for example, two to four doses per day. It will be understood, however, that the specific dose level for any particular patient will depend on a variety of factors, including the body weight, general health, diet, time and route of administration and combination with other drugs and the severity of the disease being treated. It is expected that the adult human daily dosage will normally range from between about one microgram to about one gram, preferably from between about 10 mg and 100 mg, of the nitrogen-containing virus-inhibiting compound per kilogram body weight. Of course, the amount of the composition which should be administered to a cell or animal is dependent upon numerous factors well understood by one of skill in the art, such as the molecular weight of the nitrogen-containing virus-inhibiting compound, the route of administration, and the like.

Pharmaceutical compositions that are useful in the methods of the invention may be administered systemically in oral solid formulations, ophthalmic, suppository, aerosol, topical or other similar formulations. For example, it may be in the physical form of a powder, tablet, capsule, lozenge, gel, solution, suspension, syrup, or the like. In addition to the nitrogen-containing virus-inhibiting compound, such pharmaceutical compositions may contain pharmaceutically-acceptable carriers and other ingredients known to enhance and facilitate drug administration. Other possible formulations, such as nanoparticles, liposomes, resealed erythrocytes, and immunologically based systems may also be used to administer the compound according to the method of the invention. Such pharmaceutical compositions may be administered by any known route. The term "parenteral" used herein includes subcutaneous, intravenous, intraarterial, intrathecal, and injection and infusion techniques, without limitation. By way of example, the pharmaceutical compositions may be administered orally, topically, parenterally, systemically, or by a pulmonary route.

These compositions may be administered according to the methods of the invention in a single dose or in multiple doses which are administered at different times. Because the inhibitory effect of the composition upon a virus may persist, the dosing regimen may be adjusted such that virus propagation is retarded while the host cell is minimally effected. By way of example, an animal may be administered a dose of the composition of the invention once per week, whereby virus propagation is retarded for the entire week, while host cell functions are inhibited only for a short period once per week.

The following specific examples are to be construed as merely illustrative, and not limitive, of the remainder of the disclosure.

EXAMPLES

Preparation of N-nonyl-DGJ (NN-DGJ), N-nonyl-methylDGJ (NN-MeDGJ), N-nonyl-altrostatin, N-nonyl-DNJ (NN-DNJ), N-nonyl-DMDP (NN-DMDP), and N-nonyl-2-aminobenzamide

The parent amino or imino compound (DGJ, MeDGJ, altrostatin, DNJ, DMDP, or 2-aminobenzamide (2ABC9) was reductively alkylated with nonylaldehyde (1.2 mol equivalents) in the presence of one mole equivalent of sodium cyanoborohydride for three hours at room temperature in acidified methanol. Typical yields from this reaction were greater than 95% as determined by amperometric detection after high performance cation-exchange chromatography (Dionex). N-Nonyl-compounds were purified from the reaction mixture by high performance liquid chromatography (HPLC) as follows. A sample was applied to a SCX cation-exchange column (7.5 x 50 mm) in 20% (v/v) acetonitrile and eluted with a linear gradient of 20% acetonitrile containing 500 mM ammonium formate, pH 4.4. The N-nonyl compound was recovered and applied to a C18 reverse-phase column (4.6 x 250 mm) equilibrated with 10% acetonitrile containing 0.1% trifluoroacetic acid (TFA). The compound was eluted from the column using a linear gradient of 80% acetonitrile containing 0.1% trifluoroacetic acid, lyophilized to dryness, and dissolved in methanol. Samples of purified compound were analyzed by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry using 2,5-dihydroxybenzoic acid as the matrix.

Compounds having different N-alkyl chain lengths are prepared by replacing nonyl aldehyde with the desired chain length aldehyde. Tritiated compounds are prepared by employing tritiated sodium cyanoborohydride as the reducing agent in the reaction.

(a) N-nonyl-DGJ: MALDI-TOF mass spectrometry showed a peak at 288.83 atomic mass units as expected for the structure shown in Figure 1.

(b) N-nonyl-MeDGJ: MALDI-TOF mass spectrometry showed a peak at 273.9 atomic mass units as expected for the structure shown in Figure 1.

(c) N-nonyl-altrostatin: MALDI-TOF mass spectrometry showed a peak at 289.44 atomic mass units as expected for the structure shown in Figure 1.

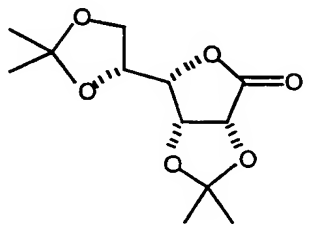
(d) N-nonyl-DMDP: MALDI-TOF mass spectrometry showed a peak at 287.66 atomic mass units as expected for the structure shown in Figure 1.

(e) N-nonyl-2-aminobenzamide (2ABC9): MALDI-TOF mass spectrometry showed a

peak at 261.57 atomic mass units as expected for the structure shown in Figure 1.

Preparation of N-(7-oxa-nonyl)-1,5,6-trideoxy-1,5-imino-D-galactitol

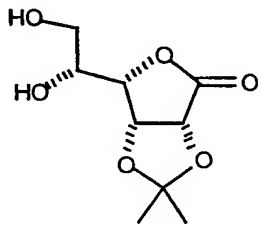
Step1: Synthesis of 2,3;5,6-Di-*O*-isopropylidene-D-gulono-1,4-lactone



p-Toluenesulfonic acid-monohydrate (1 g) was added to a stirred solution of D-gulono-lactone (20 g, 0.11 mol) in 2,2-dimethoxypropane (60 mL) and dry acetone (200 mL). After 24 hr t.l.c. (ethyl acetate) showed the consumption of starting material (R_f 0.0) and the formation of a major product (R_f 0.8). The reaction mixture was neutralized by stirring with excess sodium hydrogen carbonate, filtered and the solvent removed under reduced pressure. The residue was crystallized from ethyl acetate/hexane to give 2,3;5,6-Di-*O*-isopropylidene-D-gulono-1,4-lactone as white crystals (26.3 g, 0.1 mol, 91% yield).

M.p. 150-153°C; $[\alpha]_D^{22}$ +76.2 (c, 0.88 in acetone); δ_H (200 MHz, $CDCl_3$): 1.28 (s, 6H, $C(CH_3)_2$), 1.33, 1.37 (2 x s, 6H, $C(CH_3)_2$), 3.90 (dd, 1H, J 6.0 Hz, J 9.0 Hz), 4.02 - 4.10 (m, 1H), 4.18 - 4.27 (m, 1H), 4.49 (dd, 1H, $J_{3,4}$ 3 Hz, $J_{4,5}$ 9 Hz, H-4), 4.92 (dd, 1H, $J_{2,3}$ 6 Hz, $J_{3,4}$ 3 Hz, H-3), 4.96 (d, 1H, $J_{2,3}$ 6 Hz, H-2); δ_C (50 MHz, $CDCl_3$): 25.6 ($C(CH_3)_2$), 26.3 ($C(CH_3)_2$), 27.1 ($C(CH_3)_2$), 27.2 ($C(CH_3)_2$), 65.6 (CH_2, C-2), 75.7, 76.4, 76.5, 81.3 (4 x CH, C-2, C-3, C-4), 110.9 ($C(CH_3)_2$), 114.7 ($C(CH_3)_2$), 173.3 (C=O).

Step 2: Synthesis of 2,3-*O*-isopropylidene-D-gulono-lactone

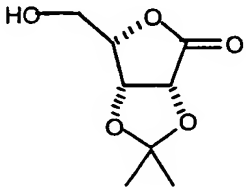


2,3;5,6-Di-*O*-isopropylidene-D-gulono-1,4-lactone (26 g, 0.1 mol) was dissolved in aquous

acetic acid (200 ml, 80%) and the solution was stirred overnight at room temperature. T.l.c. (ethyl acetate) showed the consumption of starting material (R_f 0.8) and the formation of one major product (R_f 0.4). The reaction solvent was removed and the residue crystallized from ethyl acetate/hexane to give 2,3-*O*-isopropylidene-D-gulono-1,4-lactone (20.7 g, 95 mmol, 95%) as a white solid.

M.p. 139-141°C; $[\alpha]_D^{22}$ +73.1 (c, 2.4 in acetone); δ_H (200 MHz, $CDCl_3$): 1.21, 1.22 (2 x s, 6H, $C(CH_3)_2$), 3.46-3.57 (m, 2H), 3.64-3.73 (m, 1H), 4.48 (dd, 1H, $J_{3,4}$ 5 Hz, $J_{4,5}$ 3Hz, H-4), 4.75 (d, 1H, $J_{2,3}$ 5 Hz, H-2), 4.81 (dd, 1H, $J_{2,3}$ 5 Hz, $J_{3,4}$ 3 Hz, H-3); δ_C (50 MHz, $CDCl_3$): 26.0 ($C(CH_3)_2$), 26.1 ($C(CH_3)_2$), 62.7 (CH_2, C-6), 71.3 (CH, C-3), 76.7, 77.1 (2 x CH, C-4, C-5), 81.8 (CH, C-2), 113.9 ($C(CH_3)_2$), 175.5 (C=O).

Step 3: Synthesis of 2,3-*O*-isopropylidene-L-lyxono-1,4-lactone

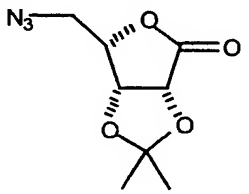


2,3-*O*-isopropylidene-D-gulonolactone (10.9 g, 50 mmol) was dissolved in dry THF (250 mL) under N_2. Periodic acid (12.8 g, 56 mmol, 1.12 eq) was added. After 5 min, the solution became cloudy and was vigorously stirred for another 15 min. The reaction mixture was purified by elution through a silica plug eluted with ethyl acetate. The solvent was removed under reduced pressure to afford a yellow oil which was dissolved in acetic acid (150 ml). Sodium cyanoborohydride (3.22 g, 51 mmol) was added and the solution stirred for 90 min. Saturated aqueous ammonium chloride solution (20 mL) was added to quench the reaction mixture and the solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate (200 mL) and washed with saturated aqueous ammonium chloride solution (50 ml), water (50 mL) and brine (50 mL). The aqueous layer was re-extracted with ethyl acetate (3 x 50 mL). The organic fractions were combined, dried (magnesium sulphate), filtered and the solvent removed. Purification by flash chromatography (ethyl acetate) gave 2,3-*O*-isopropylidene-L-lyxono-1,4-lactone (7.93 g, 42 mmol, 84% yield) as a white crystalline solid.

M.p. 94-95°C; $[\alpha]_D^{23}$ - 90.8 (c, 1.08 in acetone); δ_H (500 MHz, $CDCl_3$): 1.41, 1.49 (6H, 2 x s,

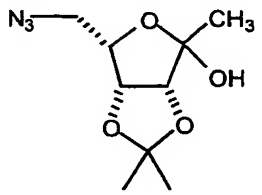
$C(CH_3)_2$), 2.18 (1H, br, OH), 3.87 (1H, dd, $J_{4,5'}$ 5.3 Hz, $J_{5,5'}$ 12.3 Hz, H-5'), 4.15 (1H, dd, $J_{4,5}$ 6.4 Hz, $J_{5,5'}$ 12.3 Hz, H-5), 4.56 (1H, ddd, $J_{4,5'}$ 5.3 Hz, $J_{4,5}$ 6.6 Hz, $J_{3,4}$ 3.6 Hz, H-4), 4.82 (1H, d, $J_{2,3}$ 5.5 Hz, H-2), 4.85 (1H, dd, $J_{3,4}$ 3.6 Hz, $J_{2,3}$ 5.5 Hz, H-3), δ_C (50 MHz, $CDCl_3$): 26.2 ($C(CH_3)_2$), 27.1 ($C(CH_3)_2$), 61.3 (CH_2, C-5), 76.6, 76.7, 79.8 (3 x CH, C-2, C-3, C-4), 114.9 ($C(CH_3)_2$), 174.3 (C=O).

Step 4: Synthesis of 5-azido-5-deoxy-2,3-*O*-isopropylidene-L-lyxono-1,4-lactone



2,3-*O*-isopropylidene-L-lyxono-1,4-lactone (5.8 g, 30.9 mmol) was dissolved in anhydrous dichloromethane (140 mL) under N_2. The solution was cooled to -30°C and dry pyridine (12 mL) was added. Trifluoromethanesulphonic anhydride (6.5 ml, 38.7 mmol) was then added dropwise to the solution which was stirred at -30°C. After 60 min, t.l.c. (ethyl acetate/hexane 1:1) showed a complete reaction. The solution was allowed to warm to 0°C and dry DMF (250 ml) and sodium azide (8.2 g, 126 mmol, 4 eq) were added. The suspension was stirred at room temperature for 4 Water (25 mL) was added to quench the reaction. The solvent was then removed under reduced pressure and co-evaporated with toluene. The residue was dissolved in dichloro methane (250 mL) and washed water (2 x 50 mL) and brine (50 mL). The aquous layer was re-extracted with dichloro methane (3 x 50 mL). The organic fractions were combined, dried (magnesium sulphate), filtered and the solvent removed. Purification by flash chromatography (hexane/ethyl acetate 1:1) afforded 5-azido-5-deoxy-2,3-*O*-isopropylidene-L-lyxono-1,4-lactone (5.8 g, 27.2 mmol, 88% yield) as white crystals.
$[\alpha]_D^{23}$ -71.0 (c, 2.0 in $CHCl_3$); υ_{max} (film/cm^{-1}) 1784 (C=O), 2101 (N_3); δ_H (500 MHz, $CDCl_3$): 1.42, 1.50 (6H, 2 x s, $C(CH_3)_2$), 3.66 (1H, dd, $J_{4,5'}$ 6.3 Hz, $J_{5,5'}$ 12.9 Hz, H-5'), 3.72 (1H, dd, $J_{4,5}$ 7.1 Hz, $J_{5,5'}$ 12.9 Hz, H-5), 4.62 (1H, ddd, $J_{4,5'}$ 6.3 Hz, $J_{4,5}$ 7.1 Hz, $J_{3,4}$ 3.5 Hz, H-4), 4.83 (1H, dd, $J_{3,4}$ 3.5 Hz, $J_{2,3}$ 5.4 Hz, H-3), 4.86 (1H, d, $J_{2,3}$ 5.4 Hz, H-2); δ_C (50 MHz, $CDCl_3$): 26.3 ($C(CH_3)_2$), 26.5 ($C(CH_3)_2$), 50.4 (CH_2, C-5), 76.1, 76.4, 77.6 (3 x CH, C-2, C-3, C-4), 115.1 ($C(CH_3)_2$), 173.4 (s, C=O); *m/z* (CI, NH_3): 218 (100%), 186 (35%, MH^+-N_2); (Found: C, 45.26; H, 5.43; N, 19.24. $C_8H_{11}O_4N_3$ requires: C, 45.07; H, 5.20; N, 19.71%).

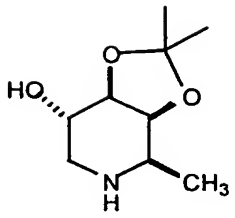
Step 5: Synthesis of 6-Azido-1,6-dideoxy-3,4-*O*-isopropylidene-L-lyxo-2,5-hexulose



5-Azido-5-deoxy-2,3-*O*-isopropylidene-L-lyxono-1,4-lactone (4 g, 18.8 mmol) was dissolved in dry THF (70 mL) under N_2 in presence of molecular sieves (4Å). The solution was cooled to -78°C. Methyl lithium (18 ml, 25.2 mmol, 1.4 M solution in diethyl ether) was added and the solution stirred at -78°C. After two hours, t.l.c. (ethyl acetate/hexane 1:1) showed no starting material (Rf 0.62) and a new product (Rf 0.72). Saturated aqueous ammonium chloride solution (10 mL) was added and the solution was stirred for 30 min. The reaction mixture was then extracted with dichloromethane (4 x 50 mL). The organic extracts were combined, dried (magnesium sulphate), filtered off and the solvent removed under reduced pressure. The resulting yellow solid was purified by flash chromatography (ethyl acetate/hexane 1:2) to give 6-azido-1,6-dideoxy-3,4-*O*-isopropylidene-L-lyxo-2,5-hexulose (3.49 g, 91% yield) as a white solid.

M.p. 89-90°C; $[\alpha]_D^{21}$ -12.5 (c, 1.01 in $CHCl_3$); ν_{max} (KBr)/cm^{-1}: 3436 (br, OH), 2101 (N_3); δ_H (500 MHz, $CDCl_3$): 1.33, 1.48 (6H, 2 x s, $C(CH_3)_2$), 1.54 (3H, s, CH_3), 2.13 (1H, br, OH), 3.54 (2H, d, $J_{6',6}$ 6.4 Hz, H-6, H-6'), 4.23 (1H, app. dt, $J_{5,4}$ 3.9 Hz, $J_{5,6}$ 6.4 Hz, H-5), 4.48 (1H, d, $J_{3,4}$ 5.9 Hz, H-3), 4.78 (1H, dd, $J_{4,3}$ 5.9 Hz, $J_{4,5}$ 3.9 Hz, H-4); δ_C (50 MHz, $CDCl_3$): 22.9 (CH_3, C-1), 25.2, 26.5 (2 x CH_3, $C(CH_3)_2$), 50.4 (CH_2, C-6), 77.9, 80.9, 85.8 (3 x CH, C-3, C-4, C-5), 105.9 (C-2), 113.4 ($C(CH_3)_2$); *m/z* (APCI+): 216 (92%), 202 (MH^+-N_2, 38%), 184 (MH^+-H_2O-N_2, 100%); (Found: C, 47.38; H, 6.53; N, 18.03%; $C_9H_{15}O_4N_3$ requires C, 47.16; H, 6.60; N, 18.33%).

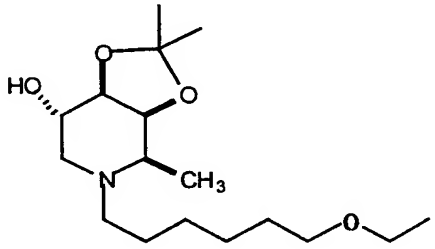
Step 6: Synthesis of 1,5,6-trideoxy-1,5-imino-3,4-O-isopropylidene-D-galactitol



6-Azido-1,6-dideoxy-3,4-*O*-isopropylidene-L-lyxo-2,5-hexulose (1.0 g, 4.4 mmol) was dissolved in ethanol (25 mL). Palladium black (300 mg) was added. The solution was degased 3 times and air was replaced by H_2. The solution was stirred at room temperature under an atmosphere of H_2. After 24 hr, the solution was filtered through a celite plug eluted with ethanol. The solvent was removed under reduced pressure to give a yellow solid which was purified by flash chromatography (chloroform/methanol 4:1) to afford 1,5,6-trideoxy-1,5-imino-3,4-O-isopropylidene-D-galactitol as a white solid (700 mg, 3.7 mmol, 84% yield).

M.p. 164-166°C; $[\alpha]_D^{22}$ +84.0 (c, 1.01 in $CHCl_3$); ν_{max} (cm^{-1}): 3434 (br, OH, NH); δ_H (500 MHz, $CDCl_3$): 1.27 (3H, d, $J_{5,6}$ 6.3 Hz, CH_3), 1.38, 1.55 (6H, 2 x s, $C(CH_3)_2$), 1.95 (1H, br, OH), 2.48 (1H, dd, $J_{1a,2}$ 10.6 Hz, $J_{1e,1a}$ 13.0 Hz, H-1a), 3.08 (1H, dq, $J_{4,5}$ 2.6 Hz, $J_{5,6}$ 6.3 Hz, H-5), 3.12 (1H, dd, $J_{1e,2}$ 5.1 Hz, $J_{1a,1e}$ 13.0 Hz, H-1e), 3.67 (1H, ddd, $J_{1',2}$ 5.1 Hz $J_{1,2}$ 10.6 Hz, $J_{2,3}$ 7.1 Hz, H-2), 3.88 (1H, dd, $J_{2,3}$ 7.1 Hz, $J_{3,4}$ 5.3 Hz, H-3), 4.04 (1H, dd, $J_{4,5}$ 2.6 Hz, $J_{3,4}$ 5.3 Hz, H-4); δ_C (50 MHz, $CDCl_3$): 18.0 (CH_3, C-6), 26.7, 28.7 (2 x CH_3, $C(CH_3)_2$), 48.7 (CH_2, C-1), 51.6 (CH, C-5), 71.1, 77.0, 80.5 (3 x CH, C-2, C-3, C-4), 109.5 ($C(CH_3)_2$); *m/z* (APCI+): 188 (MH^+, 100%), 130 (19%); (Found: C, 57.26; H, 9.40; N, 7.24%. $C_9H_{17}O_3N$ requires C, 57.73; H, 9.15; N, 7.48%)

Step 7: Synthesis of *N*-nonyl-1,5,6-trideoxy-1,5-imino-3,4-*O*-isopropylidene D-galactitol

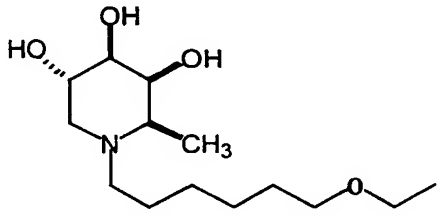


1,5,6-trideoxy-1,5-imino-3,4-*O*-isopropylidene-D-galactitol (804 mg, 4.3 mmol) was

dissolved in ethanol (15 mL). Glacial acetic acid (0.1 mL) and 6-ethoxy-hexanol (1.83 g, 12.9 mmol, 2.2 ml, 3 eq) were added. After stirring the reaction mixture for 20 min at room temperature under N_2. Palladium black (300 mg) was added. The solution was degassed three times and nitrogen was replaced by H_2. The solution was stirred at room temperature under an atmosphere of H_2. After 16 h, the solution was filtered through a celite plug eluted with ethanol (50 mL) and ethyl acetate (40 mL). The solvent was removed under reduced pressure to give a yellow solid which was purified by flash chromatography (ethyl acetate) to afford *N*-nonyl-1,5,6-trideoxy-1,5- imino-3,4-*O*-isopropylidene-D-galactitol as a white solid (829 mg, 2.7 mmol, 63% yield).

M.p. 41 - 43°C ; δ_H (200 MHz, $CDCl_3$): 0.99 (3H, t, J 7.3 Hz, CH_3), 1.22 - 1.51 (15H, 6 x CH_2, CH_3, C-6), 1.35, 1.53 (6H, 2 x s, $C(CH_3)_2$), 2.32 (1H, t, J 10.3 Hz, H-1a), 2.52 – 2.96 (m, 3H, H-5, *N*-CH_2), 3.82 – 3.94 (2H, m, H-1e, H-4); 4.12 (1H, m, H-2); δ_C (50 MHz, $CDCl_3$): 14.6 (CH_3), 16.0 (CH_3, C-6), 23.1, 24.4 (2 x CH_3, $C(CH_3)_2$), 27.9, 29.7, 29.9, 32.3 (4 x CH_2), 53.4 (CH_2, C-1), 54.1 (CH_2, N-CH_2), 55.1 (CH, C-5), 70.2, 78.1, 79.7 (3 x CH, C-2, C-3, C-4), 109.6 ($C(CH_3)_2$);

Step 8: Synthesis of *N*-(7-oxa-nonyl)-1,5,6-trideoxy-1,5-imino-D-galactitol



N-nonyl-1,5,6-trideoxy-1,5-imino-3,4-*O*-isopropylidene-D-galactitol (1.4 g, 4.5 mmol) was dissolved in 50% aqueous trifluoroacetic acid (10 mL) and the solution was stirred for two hours. The solvent was removed under reduced pressure and co-evaporated with toluene (2 x 5 mL). Purification by flash chromatography ($CHCl_3$/CH_3OH 3:1) afforded *N*-nonyl-1,5,6-trideoxy-1,5-imino-D-galactitol (1.18 g, 4.3 mmol, 96% yield);

M.p. 49-51°C; ν_{max} (cm^{-1}): 3434 (br, OH), 2845 (N-CH_2), 1672 (N-CH_2), 1203, 1133; δ_H (200 MHz, d^4-MeOH): 0.99 (3H, t, J 7.3 Hz, CH_3), 1.22 - 1.51 (15H, 6 x CH_2, CH_3, C-6), 2.88 (1H, t, J 10.6 Hz, H-1a), 3.16 (2H, m, *N*-CH_2), 3.31 (1H, m, H-5), 3.42 (1H, dd, $J_{1e,2}$ 5.0 Hz, $J_{1a,1e}$ 10.6 Hz, H-1e), 3.51 (1H, dd, $J_{4,5}$ 2.6 Hz, $J_{3,4}$ 5.3 Hz, H-4); 3.91 3.51 (1H, dd, $J_{4,5}$ 2.6 Hz, $J_{3,4}$ 5.3 Hz, H-4); 4.08 (1H, ddd, $J_{1',2}$ 5.1 Hz $J_{1,2}$ 10.6 Hz, $J_{2,3}$ 7.1 Hz, H-2), δ_C (50 MHz,

$CDCl_3$): 13.4 (CH_3), 13.6 (CH_3, C-6), 22.1, 22.7, 26.7, 29.3, 29.5, 32.0 (6 x CH_2), 52.9 (CH_2, N-CH_2), 54.2 CH_2, C-1), 60.9, 65.5, 71.9, 74.1 (4 x CH, C-2, C-3, C-4, C-5); *m/z* ($APCI^+$): 274.2 (MH^+, 100%).

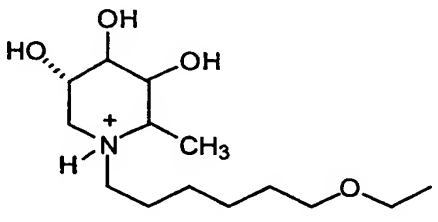
Preparation of N-7-oxa-nonyl-DGJ, N-7-oxa-nonyl-methylDGJ, N-7-oxa-nonyl-DMDP, and N-7-oxa-nonyl-2-aminobenzamide

The parent amino or imino compound (DGJ, MeDGJ, DMDP, or 2-aminobenzamide (2ABC9) was reductively alkylated with 6-ethoxy-hexanal (1.2 mol equivalents) in the presence of one mole equivalent of sodium cyanoborohydride for three hours at room temperature in acidified methanol. Typical yields from this reaction were greater than 95% as determined by amperometric detection after high performance cation-exchange chromatography (Dionex). N-7-oxa-nonyl-compounds were purified from the reaction mixture by high performance liquid chromatography (HPLC) as follows. A sample was applied to a SCX cation-exchange column (7.5 x 50 mm) in 20% (v/v) acetonitrile and eluted with a linear gradient of 20% acetonitrile containing 500 mM ammonium formate, pH 4.4. The N-7-oxa-nonyl compound was recovered and applied to a C18 reverse-phase column (4.6 x 250 mm) equilibrated with 10% acetonitrile containing 0.1% trifluoroacetic acid (TFA). The compound was eluted from the column using a linear gradient of 80% acetonitrile containing 0.1% trifluoroacetic acid, lyophilized to dryness, and dissolved in methanol. Samples of purified compound were analyzed by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry using 2,5-dihydroxybenzoic acid as the matrix.

Compounds having different N-7-oxa-alkyl chain lengths are prepared by replacing oxanonyl-aldehyde with the desired chain length aldehyde. Tritiated compounds are prepared by employing tritiated sodium cyanoborohydride as the reducing agent in the reaction.

Characterization of Synthesized Compounds

N-(7-oxa-nonyl)-1,5,6-trideoxy-1,5-imino-D-galactitol (chloride salt)



N-(7-oxa-nonyl)-1,5,6-trideoxy-1,5-imino-3,4-*O*-isopropylidene-D-galactitol (70 mg, 0.22 mmol) was dissolved in 50% aqueous trifluoroacetic acid (1 mL) and the solution was stirred for two hours. The solvent was removed under reduced pressure. Purification by flash chromatography ($CHCl_3/CH_3OH$ 3:1) afforded *N*-(7-oxa-nonyl)-1,5,6-trideoxy-1,5-imino-D-galactitol (60 mg, 0.21 mmol, 96% yield). The compound was dissolved in water (1 mL) and aqueous hydrogen chloride solution (0.18 ml, 2M, 1 eq.) was added (pH 2). The reaction mixture was stirred for three hours, after this time t.l.c. ($CHCl_3$/MeOH 4:1) showed consumption of the starting material (r_f = 0.19) and one baseline spot. The solvent was removed under reduced pressure and the remaining solid was freeze dried for 24 hr to give a yellow solid (65 mg, 0.23 mmol, 99%). The following data is for the product prior to treatment with HCl:

δ_H (200 MHz, d^4-MeOH): 1.15 (3H, t, J 7.1, CH_3), 1.39 (3H, d, J 6.5, CH_3, C-6), 1.45 - 1.81 (10H, 5 x CH_2), 2.92 (1H, t, J 10.6 Hz, H-1a), 3.02 – 3.18 (2H, m, H-1e, H-5); 3.22 - 3.62 (8H, m, N-CH_2, 2 x O-CH_2, H-2, H-4), 4.04 - 4.12 (2H, m, H-3, H-4); δ_C (50 MHz, $CDCl_3$): 13.6 (CH_3), 14.5 (CH_3, C-6), 22.0, 25.8, 26.5, 29.5 (4 x CH_2), 52.8 (CH_2, C-1), 54.2 (CH_2, N-CH_2), 61.0 (CH, C-5), 66.2, 70.4, (2 x CH_2, CH_2-O-CH_2), 65.5, 71.9, 74.1 (3 x CH, C-2, C-3, C-4); *m/z* ($APCI^+$): 276.2 (MH^+, 100%).

Toxicity of various chain length N-alkyl DNJ in MDBK cells are shown in Table 1.

TABLE 1

N-alkyl Chain Length	% Viability at 10 μM	% Viability at 100 μM
C_4	74	77
C_5	80	70
C_6	73	71
C_8	70	71
C_9	56	41
C_{10}	73	43
C_{12}	86	1
C_{16}	88	4
C_{18}	84	2

The inhibitory activity (IC_{50}) and the cell cytotoxicity (IC_{50}) of various compounds, as well as their effect on α-glucosidase and ceramide glucosyl transferase, are shown in Table 2.

TABLE 2

Compound	Inhibitor of α glucosidase	Inhibitor of glycolipid synthesis	Anti-viral effect on BVDV in MDBK cells		
			IC_{50}	CC_{50}	Selectivity index (CC_{50}/IC_{50})
DNJ	Yes	No	Yes 20 μM	ND	ND
N-butyl DNJ	Yes	Yes	Yes 60-120 μM	>>10 mM	>>100
N-nonyl DNJ	Yes	Yes	Yes 2-3 μM	250 μM	83-125
N-butyl DGJ	No	Yes	No		
N-nonyl DGJ	No	Yes	Yes 5 μM	250 μM	50
N-nonyl MeDGJ	No	No	Yes 2-3 μM	ND	ND
N-7-oxa-decyl DNJ	Yes	Yes	Yes 15-20 μM	8 mM	400-533
N-7-oxa-nonyl MeDGJ	No	No	Yes 1.5 μM	2.1 mM	1400

Note the lack of cell cytotoxicity of the N-alkyl oxa-substituted compound and its superior selectivity index.

Other Materials and Methods

Cells and transfection: CHO, MDBK and Hep G2 cells were grown in RPMI 1640 (Gibco-BRL, Rockville, MD) containing 10% fetal bovine serum (Gibco-BRL). Hep G2.2.15 cells were kindly provided by Dr. George Acs (Mt. Sinai Medical College (New York, NY) and maintained in the same manner as Hep G2 cells but with the addition of 200 μg/ml of G418 (Gibco-BRL). DNA transfection of Hep G2 cells were performed as previously described (Bruss & Ganem, *Proc. Natl. Acad. Sci. USA* 88:1059-1063, 1991). N-butyl deoxynojiricmycin (NB-DNJ) was provided by Monsanto/Searle (St. Louis, MO). N-nonyl

deoxygalactojirimycin (N-nonyl-DGJ) and N-nonyl deoxynojiricmycin (N-nonyl-DNJ) were provided by Synergy Pharmaceuticals (Somerset, NJ).

Plaque Reduction and Yield Assays: MDBK cells were grown in six-well plates in the presence or absence of inhibitor, infected with cp BVDV (moi = 0.005; 500 pfu per well) for one hour at 37°C. The inoculum was then replaced with growth medium alone or with growth media and the antiviral agent and incubated for two or three days in the presence or absence of inhibitor (plaque reduction assay). After counting the plaques by eye under the microscope, the supernatant containing secreted infectious virus was removed from the wells and used to infect a fresh monolayer of MDBK cells in six-well plates. After three days, the resulting plaques were counted under the microscope (yield assay).

Figure 5 is a bar graph showing average IC_{50} values for N-nonyl-DGJ, N-nonyl-MeDGJ, N-nonyl-DNJ, N-DMDP, N-nonyl-2-aminobenzamide (ABC9), nonylamine, and N-nonyl-altrostatin. The percent of BVDV plaques produced by an infected cell culture in the presence of different concentrations of 2ABC9 (◆), nonylamine (■), N-nonyl-altrostatin (△), N-nonyl-DGJ (×), N-nonyl-MeDGJ (ж), N-nonyl-DNJ (●), and N-nonyl-DMDP (+) are shown in Figure 6. IC_{50} values for N-nonyl-MeDGJ was less than about 2.5 μM as shown in Figure 7.

Secreted DNA analysis: Secreted DNA analysis was performed by the method of Wei et al. (*J. Virol.* 70:6455-6458, 1996). Hep G2.1.15 cells were seeded at 85-90% confluency in T-75 flasks and three days later the indicated drug added at the specified concentrations: 3TC (1 μM unless noted); N-butyl-DNJ (4.52 mM); N-nonyl DNJ (either 7 μM, 70 μM or 100 μM as noted); N-nonyl-DGJ (either 7 μM, 70 μM or 100 μM as noted). Media containing drug was changed every two days and on the 7th day the media taken and the virus concentrated by pelleting through 20% sucrose for 16 hours (SW 41 rotor, 36,000 RPM). Virus was resuspended in 400 μL of 10 mM TRIS (pH 7.9), 10 mM EDTA (pH 8.0), and 10 mM $MgCl_2$. Samples were split into two 200 μL aliquots and labeled as +Dnase and -Dnase. To both tubes, 15 μl of proteinase K was added to a final concentration of 750 μg/ml for one hour at 37°C. After one hour, 10 μl Dnase was added to the tube labeled +Dnase (final concentration is 50 units/ml) and incubated at 37°C for one hour. SDS was added to a final concentration of 1% and additional proteinase K added to a final concentration of 500 μg/ml and the reaction allowed to proceed at 37°C for 3-4 hours. DNA was than purified by

phenol/chloroform extraction. DNA was separated on 1% agarose gel and probed with ^{32}P labeled probes as described (Mehta et al., *Proc. Natl. Acad. Sci. USA* 94:1822-1827, 1997).

Intracellular DNA analysis: Hep G2.2.15 cells were either left untreated or treated with the compounds listed above for seven days and the total DNA extracted as described (Mehta et al., *ibid.*). DNA (20 μg) was digested with HindIII, resolved through a 1.2% agarose gel and transferred to nylon membranes. Membranes were then hybridized with a ^{32}P labeled probe containing the total HBV genome and developed as described (Lu et al., *Proc. Natl. Acad. Sci. USA* 94:2380-2385, 1997). The relaxed circular (rc), linear (lin), and closed circular (CCC) DNA were confirmed by enzymatic digestion.

Endogenous polymerase assay: Media containing HBV from Hep G2.2.15 cells was pelleted through 20% sucrose (SW 28 Rotor, 24,000 RPM) for 16 hours and the pellet re-suspended in 50 μl of a mixture containing 50 mM Tris (pH 7.5), 75 mM NH_4Cl, 1 mM EDTA, 25 mM $MgCl_2$, 0.1% β-mercaptoethanol, 0.5% NP-40, 0.4 mM each of dATP, dGTP, dTTP and 10 μl of P^{32} labeled dCTP. Drug was added to a final concentration of 3TC (7 μM), NB-DNJ (5 mM), NN-DNJ (100 μM) and NN-DGJ (100 μM) and the samples placed at 37°C overnight and the next day proteinase K was added to a final concentration of 500 μg/ml and incubated at 37°C for one hour. DNA was purified by a phenol/chloroform extraction and ethanol precipitation.

Secretion of Infectious BVDV in the Presence of Long Chain N-Alkyl Compounds

MDBK cells were grown to semi-confluence in individual wells of 24-well plates. The cells were then infected by BVDV by incubating the cells for one hour at 37°C in the presence of approximately 500 PFU of the NADL strain of BVDV suspended in growth medium. The inoculum was then replaced with growth medium alone or growth medium containing a particular concentration of a long chain N-alkyl compound. After three days, the supernatants were removed and used to infect fresh MDBK monolayers in six-well plates. After three days, the cell monolayers were observed microscopically before and after staining with 0.2% (w/v) crystal violet in ethanol for plaque counting, and 0.2% neutral red for viability and the presence and number of virus-induced plaques was determined. The results were expressed as percentages of the number of plaques resulting from infection with the inhibitor-free plaque assay supernatant (=100%). The results of these experiments are

presented in the graphs depicted in Figure 2, Figure 3, and Figure 4. Figure 2 is a graph depicting the variation in IC_{50} for N-alkylated DNJ compounds having the following chain lengths: butyl, pentyl, hexyl, octyl, nonyl, decyl, dodecyl, hexadecyl, and octadecyl.

Inhibitory constants for various chain length N-alkyl DNJ derivatives for ceramide glucosyl transferase (CerGlcT) and α-glucosidase are summarized in Table 3.

TABLE 3

N-alkyl Chain Length	CerGlcT (IC_{50}, μM)	α-Glucosidase (IC_{50}, μM)
C_4	34.4	0.57
C_5	26.8	
C_6	23.8	
C_8	16.8	
C_9	7.4	
C_{10}	3.1	0.48
C_{12}	5.2	
C_{16}	3.4	
C_{18}	4.1	

Uptake of radioactively labeled inhibitors by different cell types

MDBK and HepG2 cells were grown to confluency in 12-well plates and incubated in the presence of tritiated long chain N-alkylated compounds (100,000 cpm/well) for the times indicated in Figure 7. The supernatant was removed and kept. The cells were washed with PBS (2x500 μL), fixed with 500 μL of ice-cold 10% perchloric acid/2% phosphotungstic acid, washed twice with 500 μL of icecold ethanol, air dried, and lysed overnight at room temperature with 500 μL of 0.5 M NaOH. The percentage of radioactive counts in the supernatant, PBS wash and lysed cells was determined by liquid scintillation counting. The results are shown graphically in Figure 8.

Secretion of HBV in the presence of lamivudine, NN-DNJ and NN-DGJ

Hep G 2.2.15 cells are a stably transfected line of HepG2 hepatoblastama cells that contain a dimer of the HBV genome and produce and secrete infectious HBV. This is a cell line that has been used as a standard in the pre-clinical evaluation of HBV antiviral agents, as enveloped HBV can be detected in the culture medium by antigen capture methods. The ability of NN-DGJ to inhibit enveloped HBV secretion from 2.2.15 cells was compared with lamivudine (3TC) and NN-DNJ, using the antigen capture method, described previously. Briefly, 2.2.15 cells were grown to confluence and then incubated with the indicated

concentrations of compound. At 6 and 9 days after incubation in the presence of compound, the amount of enveloped HBV in the culture medium was determined by PCR amplification of viral DNA from samples obtained by immunoprecipitation with HbsAg specific antibody. The results after nine days of incubation are shown in Table 4. Medium collected after nine days of incubation contained easily detectable amounts of HBV. As expected, 3TC (lamivudine) was effective in reducing the amount of enveloped HBV in the culture medium, when compared with the untreated controls. NN-DGJ was at least as effective as NN-DNJ in reducing the HBV secretion. The IC50 values for NN-DNJ and NN-DGJ were about 1 and 0.5 μM, respectively, in this assay. MTT assays of these cultures revealed that no measurable toxicity was observed for the concentrations used and time of exposure. These results showed that NN-DGJ is effective in preventing the secretion of HBV from Hep G2.2.15 cells at micromolar concentrations.

TABLE 4: Secretion of Hepatitis B virus (HBV) from Hep G2.2.15 cells in the absence and presence of antiviral compounds

COMPOUND[1]	IC 50[2]	TOX 50[3]
3TC	5 uM	>100 uM
NN-DNJ	0.4-4 uM	>100 uM
NN-DGJ	1.5-5 uM	>200 uM

[1]2.2.15 cells were grown to confluence in 96 well trays and the amount of HBV in the culture medium determined by an antigen capture/PCR based assay after 6 and 9 days of incubation the absence or presence of three concentrations of either 3TC (lamivudine), NN-DNJ or NN-DGJ. Pairs of wells were used for each concentration point.

[2]IC 50: The concentration of compound that prevented the secretion of 50% of the amount of HBV detected in the medium from wells containing untreated cultures. IC 90s were achieved for each of the compounds used.

[3]TOX 50: The concentration of compound that reduced the amount of MTT activity to 50% of that of the untreated controls, as determined on the cultures at the conclusion of the

experiment (10 days). Note that because Tox 50s were not reached with even the highest concentrations of compounds used, values are given as ">" (more than).

Effect of N-nonyl-DGJ on secretion of HBV as measured by Southern blot hybridization

HepG2.2.15 cells were grown for seven days in the absence or presence of NB-DNJ (1000 μg/ml), NN-DNJ (20 μg/ml) or NN-DGJ (20 μg/ml), respectively. After seven days, virus was isolated from these cell cultures, concentrated, and purified. Secreted HBV DNA was detected by Southern blot hybridization. HBV viral DNA from untreated cells was readily detected. The secretion of HBV DNA from treated HepG2.2.15 was also detected. N-butyl-DNJ and N-nonyl-DNJ caused a small decrease of about 3-fold and 1.5-fold secreted virus DNA, respectively; whereas N-nonyl-DGJ showed a considerably greater reduction of about 14-fold.

Intracellular levels of HBV DNA in HepG2.2.15 cells grown in the presence of 3TC, and various iminosugars

An infected cell contains several forms of HBV DNA which represent different stages in the HBV life cycle. For example, covalently closed circular DNA (CCC DNA) is the nuclear form of the DNA and is thought to be the viral template (Heermann & Gerlich, 1992). In contrast, the relaxed circular DNA (rc DNA) and linear forms (lin) are associated with the viral particle and their presence is an indicator of encapsidation of the viral pre-genomic RNA and the subsequent reverse transcription into progeny DNA (Ganem, *Curr. Top. Microbiol. Immunol.* 168:61-83, 1991). The accumulation of intracellular HBV DNA from HepG2.2.15 cells left untreated or treated with 3TC (1 μg/ml), NB-DNJ (1000 μg/ml), NN-DNJ (2 μg/ml or 20 μg/ml), or NN-DGJ (2 μg/ml or 20 μg/ml) was determined as described above. The amount of virus associated with the cells was detected seven days later by Southern blot analysis. The locations of the HBV relaxed circular DNA (rcDNA), covalently close circular (CCC) DNA, and single stranded (SS) DNA was identified by relative mobility.

HBV relaxed circular DNA (rc DNA) is easily observed, as are the smaller replicative intermediates. Treatment with 3TC leads to a complete disappearance of intracellular HBV DNA. This is consistent with 3TC acting as a polymerase inhibitor and preventing DNA production (Doong et al., *Proc. Natl. Acad. Sci. USA* 88:8495-8499, 1991). In contrast, treatment with N-butyl-DNJ causes a dramatic increase in the replicative forms of HBV DNA

(Mehta et al., *Proc. Natl. Acad. Sci. USA* 94:1822-1827, 1997). This finding is consistent with the action of this drug in preventing viral envelopment and budding but having no direct effect on DNA synthesis. Surprisingly, N-nonyl-DNJ did not cause a large increase in intracellular HBV DNA but rather a reduction. This reduction was even more pronounced with N-nonyl-DGJ, leading to an almost complete disappearance of intracellular HBV DNA (greater than 25 fold). This result clearly differentiates the action of N-nonyl-DNJ and N-nonyl-DGJ from N-butyl-DNJ.

Effect of lamivudine and iminosugars on HepG2.2.15 polymerase activity

HBV DNA replication involves the conversion of a pregenomic RNA (pgRNA) into DNA by the action of the HBV polymerase. Current nucleoside analogue drugs (e.g., 3TC) for treating HBV target this reaction, preventing the formation and secretion of HBV viral DNA. Because the iminosugar N-nonyl-DGJ prevents the formation of HBV rc DNA, it was important to determine whether N-nonyl-DGJ was acting by inhibiting the elongation step of the polymerase. HBV virions from normal and drug treated Hep G2.2.15 cells were purified and the endogenous polymerase activity was measured. HBV virions were purified from the culture medium of untreated cells by ultracentrifugation and the polymerase activity (in the presence of the indicated compounds) tested by the method of Ganem et al. (1998). Briefly, partially purified viral particles were incubated overnight with the indicated concentrations of compound and 10 μCi of ^{32}P-dCTP. Viral DNA was purified by phenol extraction and ethanol precipitation and resolved on a 1.2% agarose gel. The gel was dried and viral DNA bands detected using a PhosphoImager.

The activity of polymerase from untreated virons was measured by incorporation of radioactive nucleotides into rc DNA. In contrast, treatment with 3TC (20 μM) inhibited polymerase activity. This is consistent with 3TC acting as a polymerase inhibitor. N-butyl-DNJ (4.52 mM) showed no effect on polymerase activity, consistent with its mechanism as an α-glucosidase inhibitor. Both N-nonyl-DNJ (69 μM) and N-nonyl-DGJ (69 μM) also had no effect on polymerase activity, although both these drugs were shown above to cause a significant decrease in intercellular HBV DNA levels. These data suggest that these alkyl chain derivatives must inhibit the formation or stability of the HBV DNA by an alternative method than inhibition of polymerase activity.

All cited publications, books, patents, and patent applications are incorporated by reference in their entirety where they are cited including the priority documents U.S. Appln.

No. 60/148,101 filed August 10, 1999 and U.S. Appln. No. 60/198,621 filed April 20, 2000.

From the foregoing, it would be apparent to persons skilled in the art that the invention can be embodied in other specific forms without departing from its spirit or essential characteristics. For example, all combinations of the embodiments described above are considered part of the invention with the proviso that the prior art is excluded. The described embodiments should be considered only as illustrative, not restrictive, because the scope of the invention will be indicated by the appended claims rather than by the foregoing description. All modifications which come within the meaning and range of the lawful equivalency of the claims are to be embraced within their scope. In that sense, no particular order of process steps is intended unless explicitly recited.

CLAIMS

1. A method of inhibiting morphogenesis of a pestivirus or a flavivirus comprising administering an effective amount of a nitrogen-containing virus-inhibiting compound, or a pharmaceutically acceptable salt thereof, to a cell or an individual infected with said virus, wherein said nitrogen-containing virus-inhibiting compound is comprised of an N-C_8-C_{16} alkyl group or an oxa-substituted derivative thereof with the proviso that said nitrogen-containing virus-inhibiting compound is not N-nonyl-1,5-deoxy-1,5-imino-D-glucitol (N-nonyl-DNJ).

2. The method of claim 1, wherein the nitrogen-containing virus-inhibiting compound includes an N-C_8-C_{10} alkyl group or an oxa-substituted derivative thereof.

3. The method of claim 2, wherein the nitrogen-containing virus-inhibiting compound is N-nonyl-1,5-dideoxy-1,5-imino-D-galactitol (N-nonyl DGJ) or N-nonyl-1,5,6-trideoxy-1,5-imino-D-galactitol (N-nonyl MeDGJ).

4. The method of claim 2, wherein the nitrogen-containing virus-inhibiting compound includes an N-oxa-nonyl group.

5. The method of any one of claims 1-4, wherein the nitrogen-containing virus-inhibiting compound is selected from the group consisting of N-alkylated piperidines, N-alkylated pyrrolidines, N-alkylated phenylamines, N-alkylated pyridines, N-alkylated pyrroles, N-alkylated amino acids, and oxa-substituted derivatives thereof.

6. The method of claim 5, wherein the nitrogen-containing virus-inhibiting compound is an N-alkylated piperidine, N-alkylated pyrrolidine, or oxa-substituted derivative thereof which is an imino sugar.

7. The method of any one of claims 1-4, wherein the nitrogen-containing virus-inhibiting compound has an IC_{50} of about 20 μM or less for inhibition of hepatitis B virus.

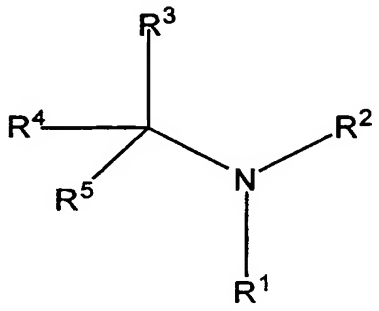
8. The method of any one of claims 1-4, wherein the nitrogen-containing virus-inhibiting compound has an IC_{50} of about 5 μM or less for inhibition of hepatitis B virus.

9. The method of any one of claims 1-4, wherein the nitrogen-containing virus-inhibiting compound has an IC_{50} of about 20 μM or less for inhibition of hepatitis B virus.

10. The method of any one of claims 1-4, wherein the nitrogen-containing virus-inhibiting compound has an IC_{50} of about 5 μM or less for inhibition of bovine viral diarrhea virus.

11. The method of any one of claims 1-10, wherein the nitrogen-containing virus-inhibiting compound does not inhibit α-glucosidase and ceramide glucosyl transferase as well as N-nonyl-DNJ.

12. The method of claim 1, wherein the nitrogen-containing virus-inhibiting compound has the formula:



wherein:

R^1 is a C_8-C_{16} alkyl or an oxa-substituted derivative thereof;

R^2 is hydrogen, R^3 is carboxy or a C_1-C_4 alkoxycarbonyl, or R^2 and R^3, together, are

$-(C)_n-$ or $-(CXY)_n-$, wherein n is 3 or 4, each X, independently, is selected from the group consisting of hydrogen, hydroxy, amino, carboxy, C_1-C_4 alkylcarboxy, C_1-C_4 alkyl, C_1-C_4 alkoxy, C_1-C_4 hydroxyalkyl, C_1-C_6 acyloxy, and aroyloxy, and each Y, independently, is selected from the group consisting of hydrogen, hydroxy, amino, carboxy, C_1-C_4

alkylcarboxy, C_1-C_4 alkyl, C_1-C_4 alkoxy, C_1-C_4 hydroxyalkyl, C_1-C_6 acyloxy, aroyloxy, and deleted;

R^4 is hydrogen or deleted; and

R^5 is selected from the group consisting of hydrogen, hydroxy, amino, substituted amino, carboxy, alkoxycarbonyl, aminocarbonyl, alkyl, aryl, aralkyl, alkoxy, hydroxyalkyl, acyloxy, and aroyloxy, or R^3 and R^5, together, form a phenyl and R^4 is deleted; wherein when R^2 and R^3, together, are $-(CXY)_n-$ and R^4 is deleted, all Y are deleted, or a physiologically acceptable salt or solvate of said compound.

13. The method of claim 12, wherein R^1 is a C_8-C_{10} alkyl or an oxa-substituted derivative thereof.

14. The method of claim 13, wherein R^2 is hydrogen, R^3 is carboxy or C_1-C_4 alkoxycarbonyl, R^4 is hydrogen, and R^5 is selected from the group consisting of hydrogen, hydroxy, amino, substituted amino, carboxy, alkoxycarbonyl, aminocarbonyl, alkyl, aryl, aralkyl, alkoxy, hydroxyalkyl, acyloxy, and aroyloxy.

15. The method of claim 14, wherein R^3 is carboxy.

16. The method of claim 14, wherein R^3 and R^5, together, form a phenyl and R^4 is deleted.

17. The method of claim 12 or claim 13, wherein R^2 and R^3, together, are $-(CXY)_n-$, wherein n is 3 or 4, each X and each Y, independently, is selected from the group consisting of hydrogen, hydroxy, amino, carboxy, C_1-C_4 alkylcarboxy, C_1-C_4 alkyl, C_1-C_4 alkoxy, C_1-C_4 hydroxyalkyl, C_1-C_6 acyloxy, and aroyloxy.

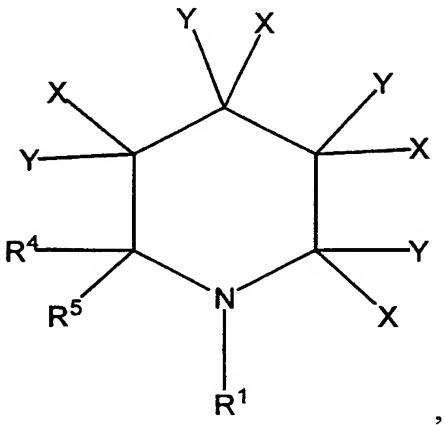
18. The method of claim 17, wherein each X is hydrogen and each Y, independently, is selected from the group consisting of hydroxy, C_1-C_4 alkyl, C_1-C_4 alkoxy, C_1-C_4 hydroxyalkyl, C_1-C_6 acyloxy, and aroyloxy.

19. The method of claim 18, wherein R^4 is hydrogen and R^5 is hydrogen.

20. The method of claim 13, wherein R^4 is deleted and R^2 and R^3, together, are $-(CXY)_n-$, wherein n is 3 or 4, each Y is deleted, and each X, independently, is selected from the group consisting of hydrogen, hydroxy, amino, carboxy, C_1-C_4 alkylcarboxy, C_1-C_4 alkyl, C_1-C_4 alkoxy, C_1-C_4 hydroxyalkyl, C_1-C_6 acyloxy, and aroyloxy.

21. The method of claim 13, wherein each X, independently, is selected from the group consisting of hydrogen, hydroxy, C_1-C_4 alkyl, C_1-C_4 alkoxy, C_1-C_4 hydroxyalkyl, C_1-C_6 acyloxy, and aroyloxy.

22. The method of claim 12, wherein the nitrogen-containing virus-inhibiting compound has the formula:



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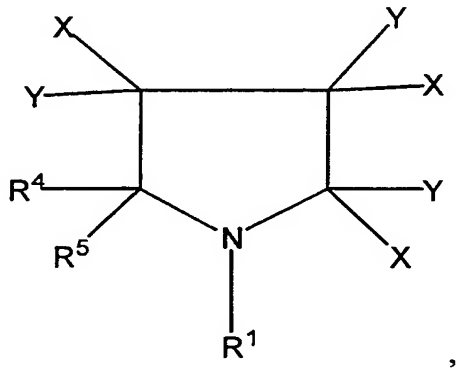

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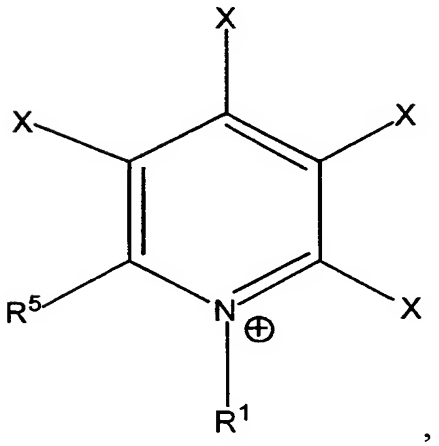

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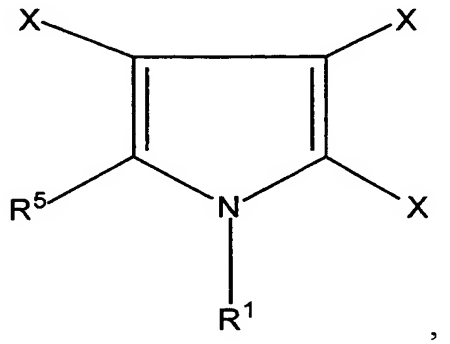
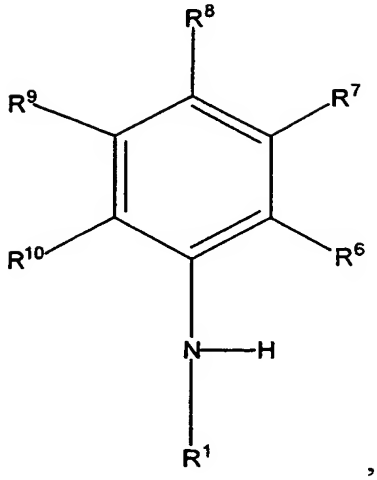
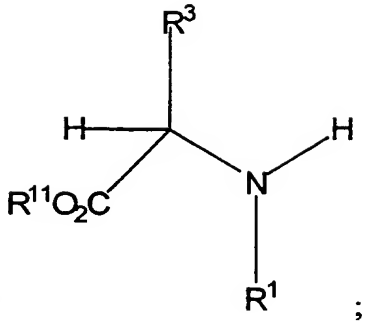


,



, or

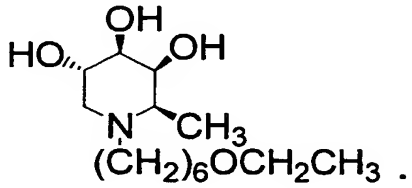


;

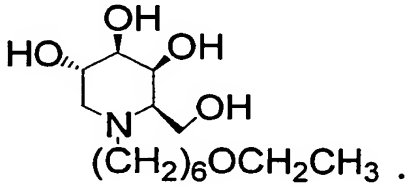
wherein each of R^6-R^{10}, independently, is selected from the group consisting of hydrogen, hydroxy, amino, carboxy, C_1-C_4 alkylcarboxy, C_1-C_4 alkyl, C_1-C_4 alkoxy, C_1-C_4 hydroxyalkyl, C_1-C_4 acyloxy, and aroyloxy; and R^{11} is hydrogen or C_1-C_6 alkyl.

23. The method of claim 1, wherein the nitrogen-containing virus-inhibiting compound is selected from the group consisting of N-nonyl altrostatin, N-nonyl-*2R,5R*-dihydroxymethyl-*3R,4R*-dihydroxypyrrolidine (N-nonyl DMDP), and N-nonyl-2-aminobenzamide (2ABC9).

24. The method of claim 1, wherein the nitrogen-containing virus-inhibiting compound is N-(7-oxa-nonyl)-1,5,6-trideoxy-1,5-imino-D-galactitol (N-7-oxa-nonyl MeDGJ)



25. The method of claim 1, wherein the nitrogen-containing virus-inhibiting compound is N-(7-oxa-nonyl)-1,5-dideoxy-1,5-imino-D-galactitol (N-7-oxa-nonyl DGJ)



26. The method of any one of claims 1-25, wherein a mammalian cell is treated.

27. The method of any one of claims 1-25, wherein a human cell is treated.

28. The method of any one of claims 1-25, wherein a mammal is treated.

29. The method of any one of claims 1-25, wherein a human is treated.

30. The method of any one of claims 1-25, wherein the virus is a hepatitis B virus.

31. The method of any one of claims 1-25, wherein the virus is a hepatitis C virus.

32. A compound having the formula shown in claim 12 or a physiologically acceptable salt or solvate of said compound.

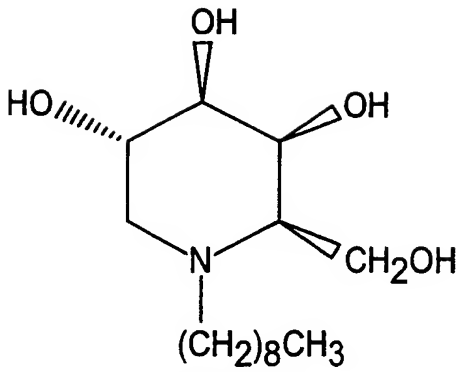
33. The compound of claim 32, wherein the compound is selected from the group consisting of N-nonyl-1,5-dideoxy-1,5-imino-D-galactitol (N-nonyl DGJ) N-nonyl-1,5,6-trideoxy-1,5-imino-D-galactitol (N-nonyl MeDGJ), and physiologically acceptable salts or solvates thereof.

34. The compound of claim 32, wherein the compound is selected from the group consisting of N-nonyl altrostatin, N-nonyl DMDP, N-nonyl-2-aminobenzamide, and physiologically acceptable salts or solvates thereof.

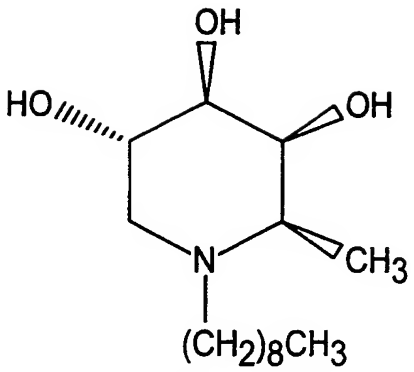
35. The compound of claim 32, wherein the compound is selected from the group consisting of N-(7-oxa-nonyl)-1,5,6-trideoxy-1,5-imino-D-galactitol (N-7-oxa-nonyl MeDGJ), N-(7-oxa-nonyl)-1,5-dideoxy-1,5-imino-D-galactitol (N-7-oxa-nonyl DGJ), and physiologically acceptable salts or solvates thereof.

36. A pharmaceutical composition comprising a nitrogen-containing virus-inhibiting compound and a pharmaceutically acceptable carrier, wherein the nitrogen-containing virus inhibiting compound includes an N-C_8-C_{16} alkyl group.

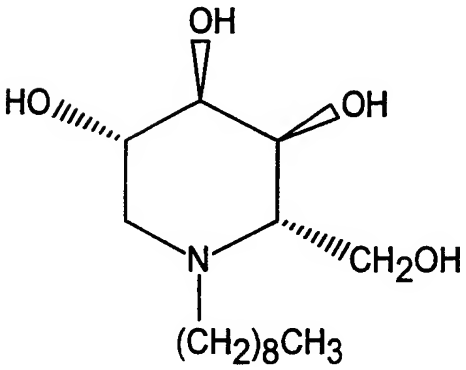
37. A method of manufacturing a pharmaceutical composition comprising combining a nitrogen-containing virus-inhibiting compound with a pharmaceutically acceptable carrier, wherein the nitrogen-containing virus inhibiting compound includes an N-C_8-C_{16} alkyl group.



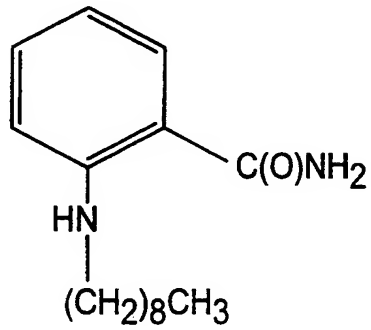
N-Nonyl-DGJ



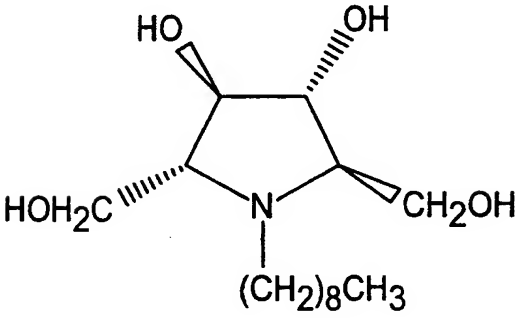
N-nonyl-MeDGJ



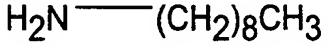
N-nonyl-altrostatin



N-nonyl-2-aminobenzamide (2ABC9)



N-nonyl-DHDP



nonylamine

FIG. 1

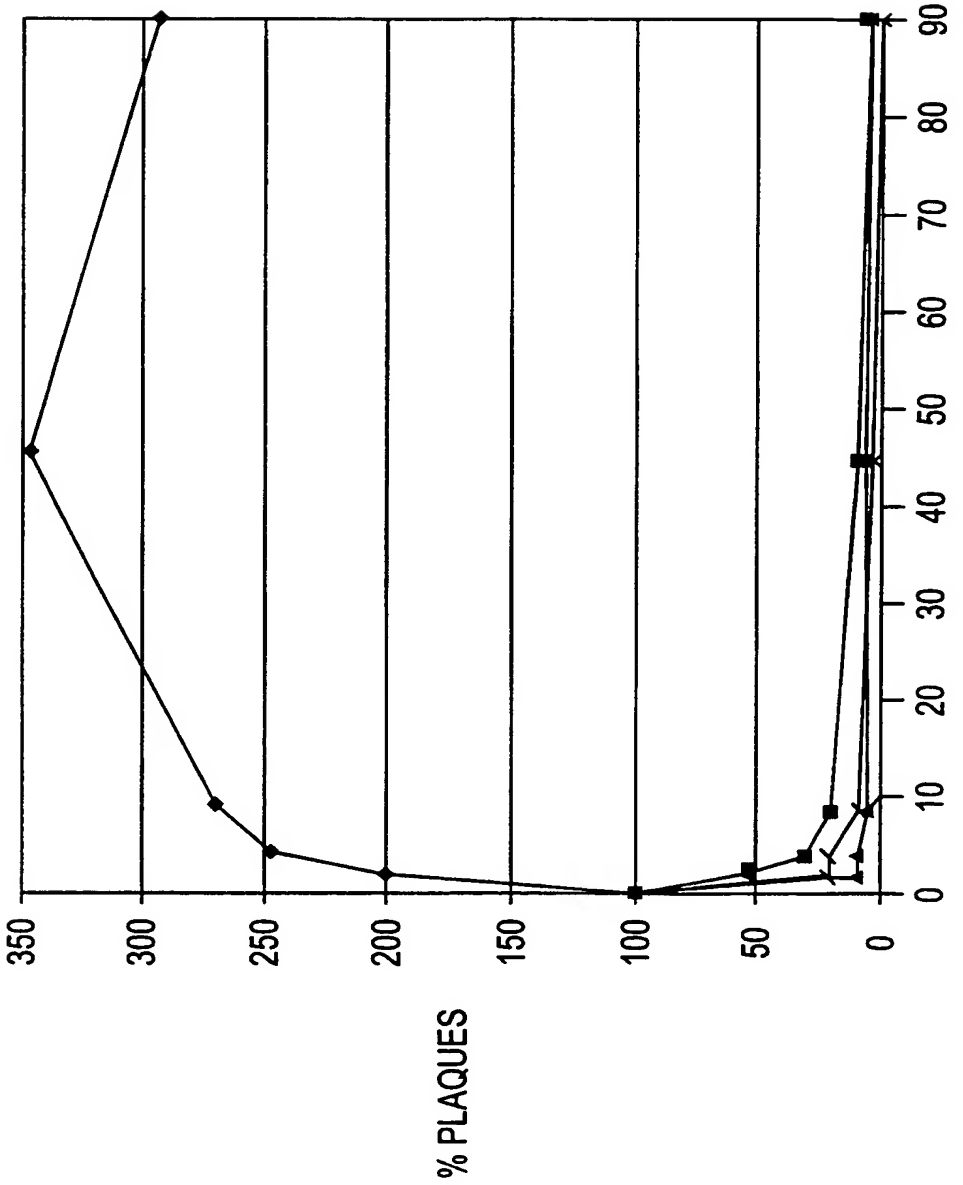


FIG. 2

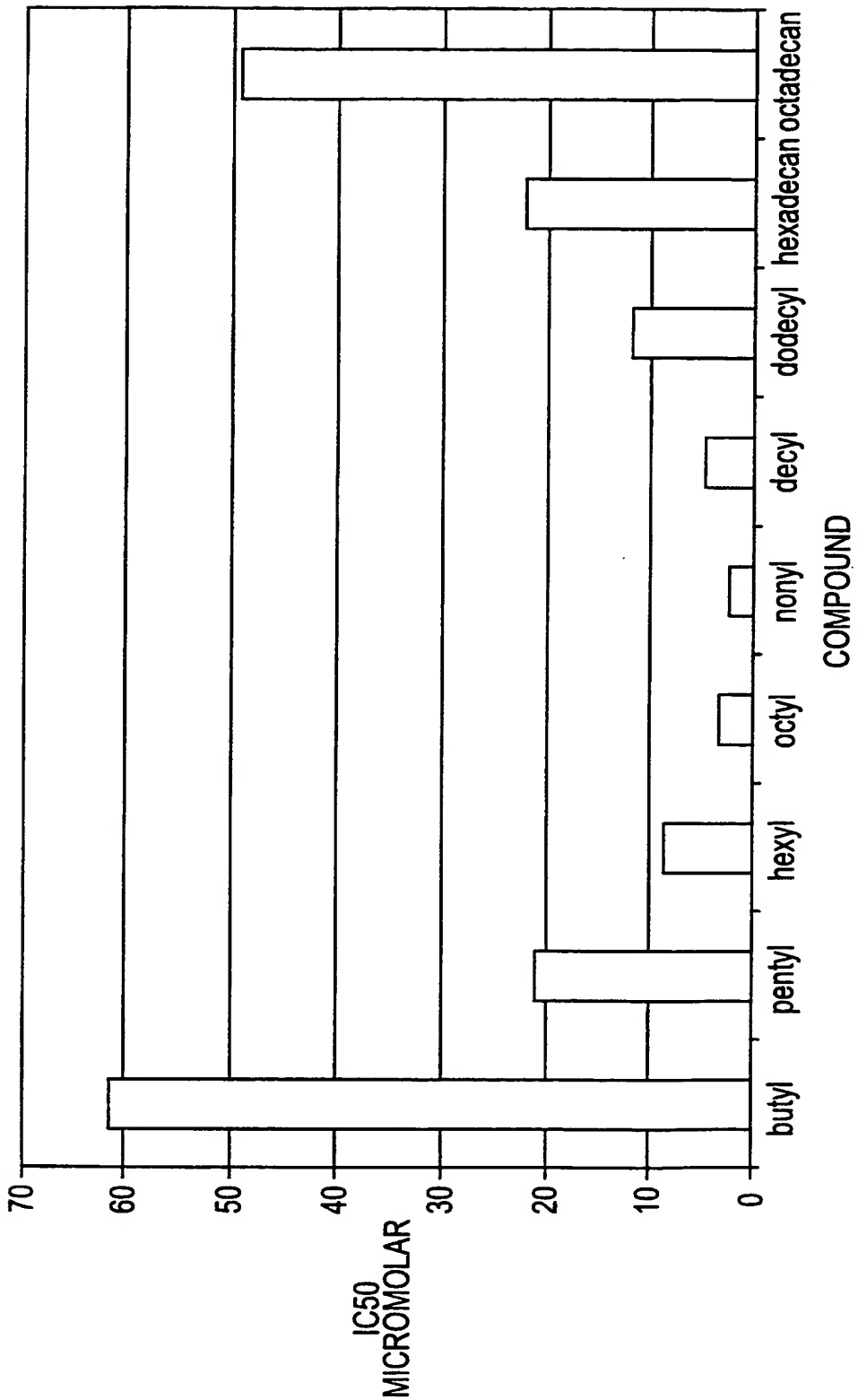

70
60
50
40
30
20
10
0
IC50
MICROMOLAR
butyl
pentyl
hexyl
octyl
nonyl
decyl
dodecyl
hexadecan
octadecan
COMPOUND

FIG. 3

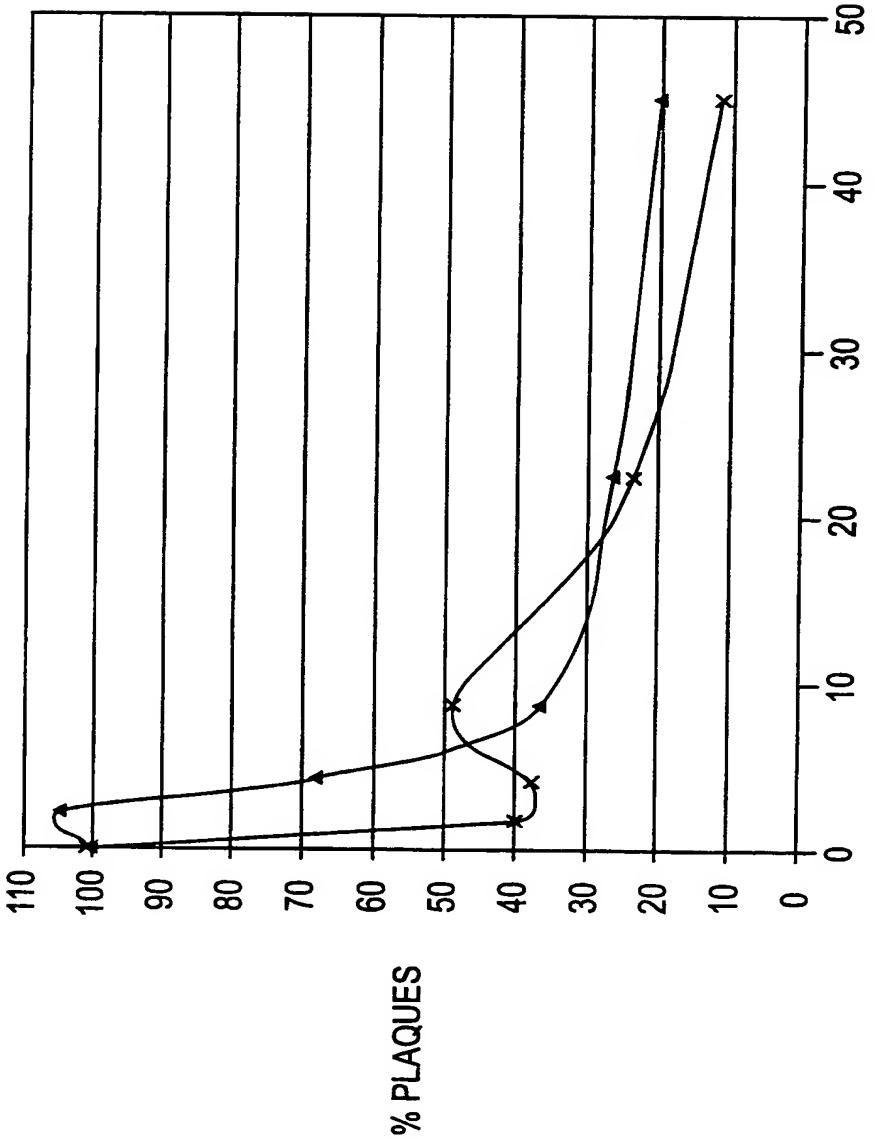


FIG. 4

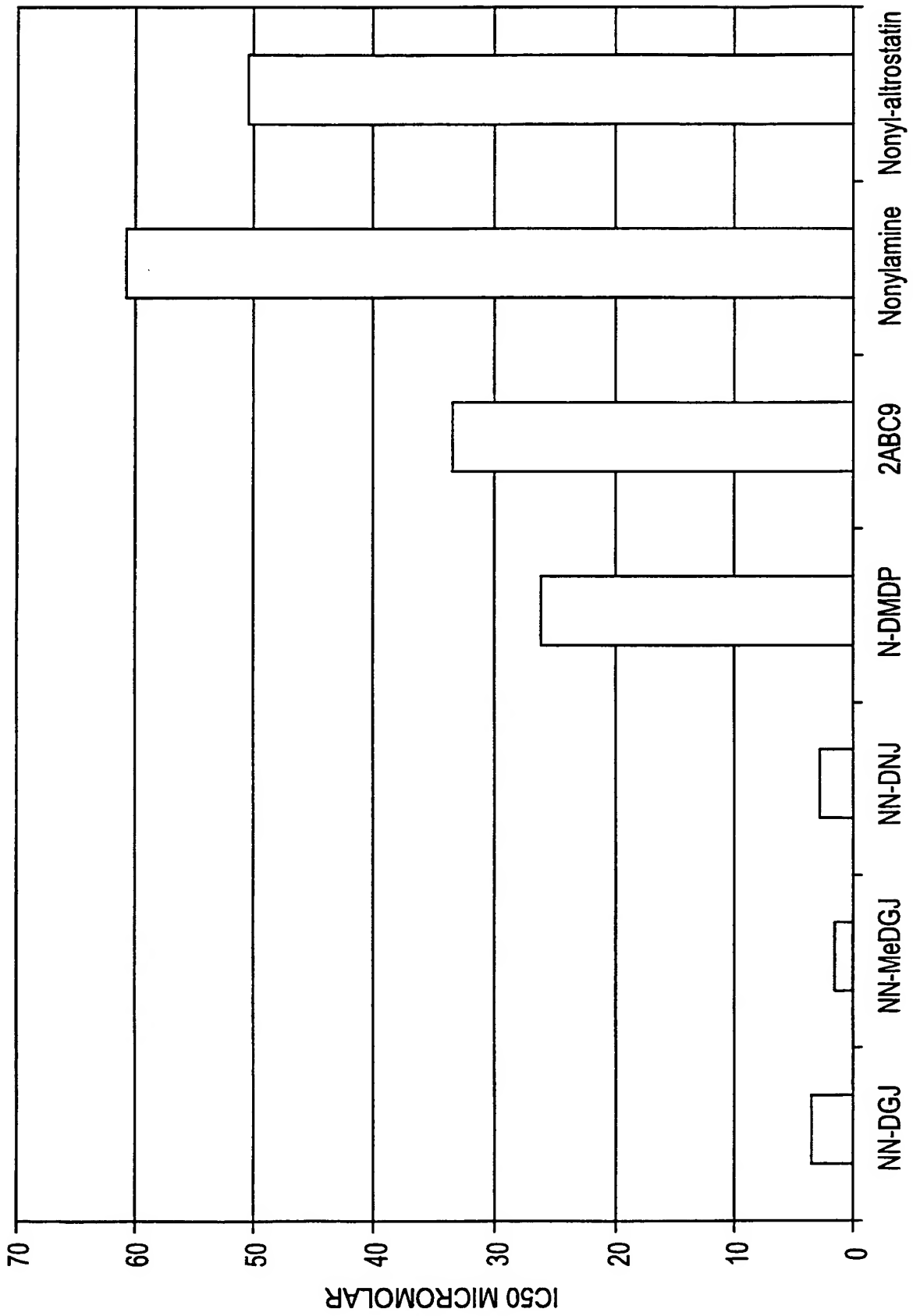

IC50 MICROMOLAR
0
10
20
30
40
50
60
70
NN-DGJ
NN-MeDGJ
NN-DNJ
N-DMDP
2ABC9
Nonylamine
Nonyl-altrostatin

FIG. 5

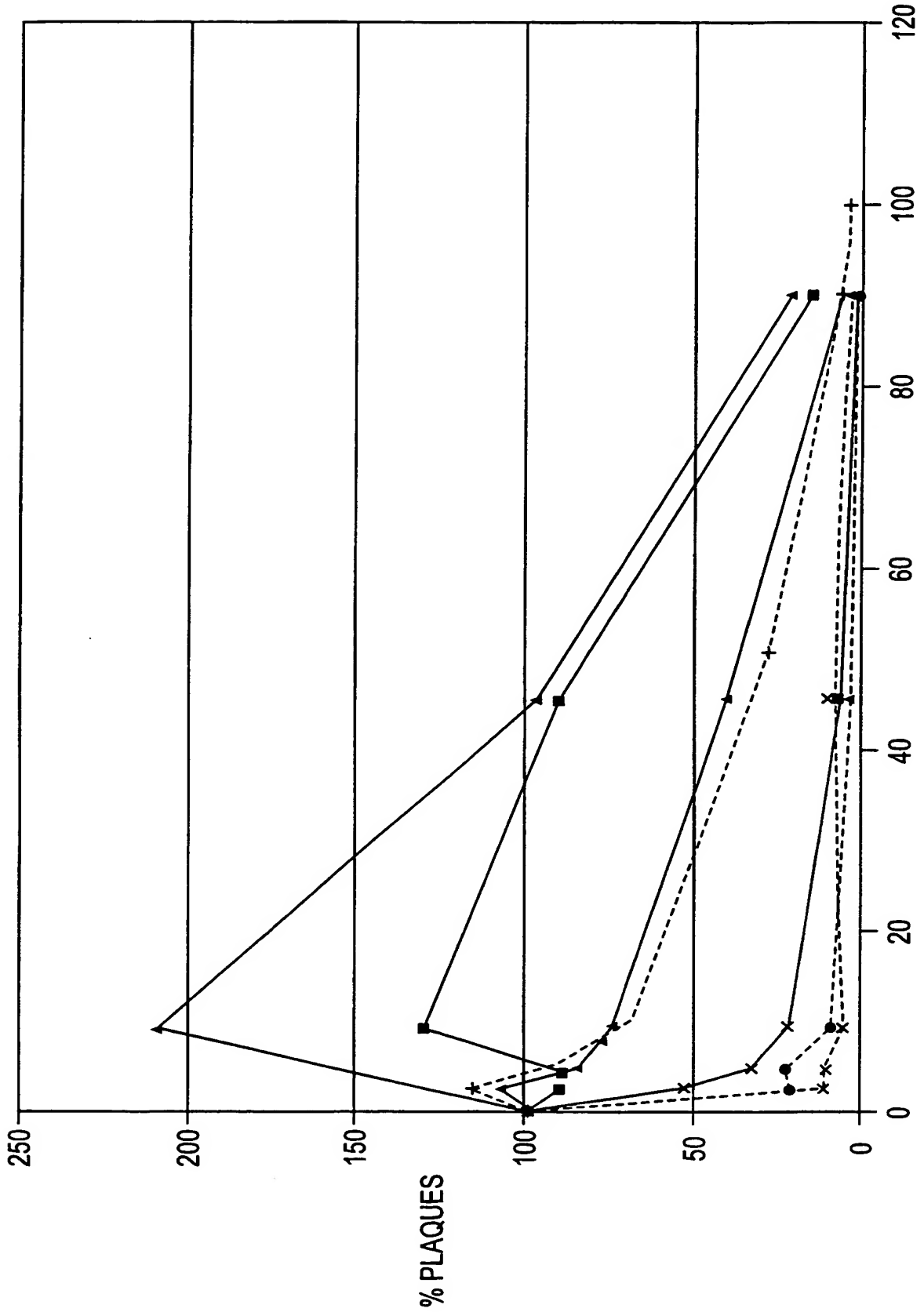


FIG. 6

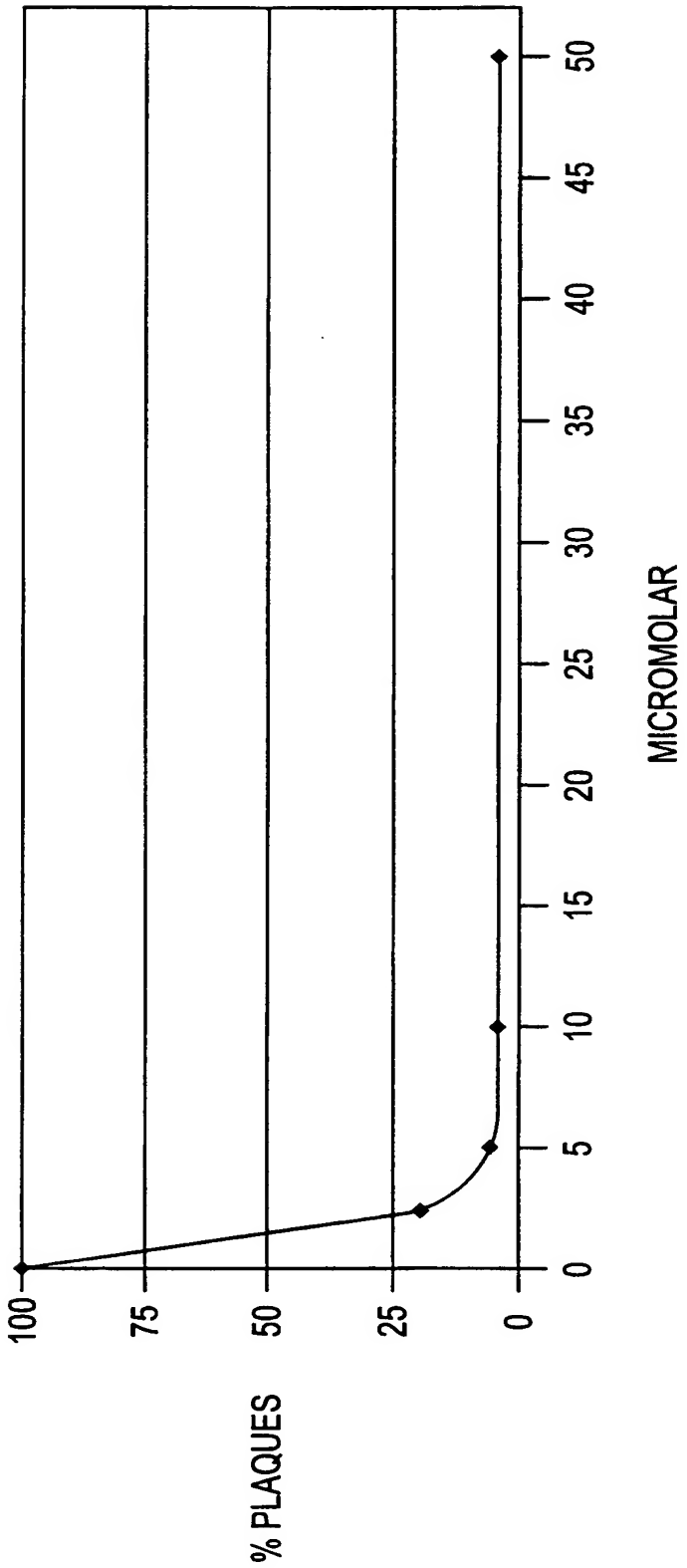


FIG. 7

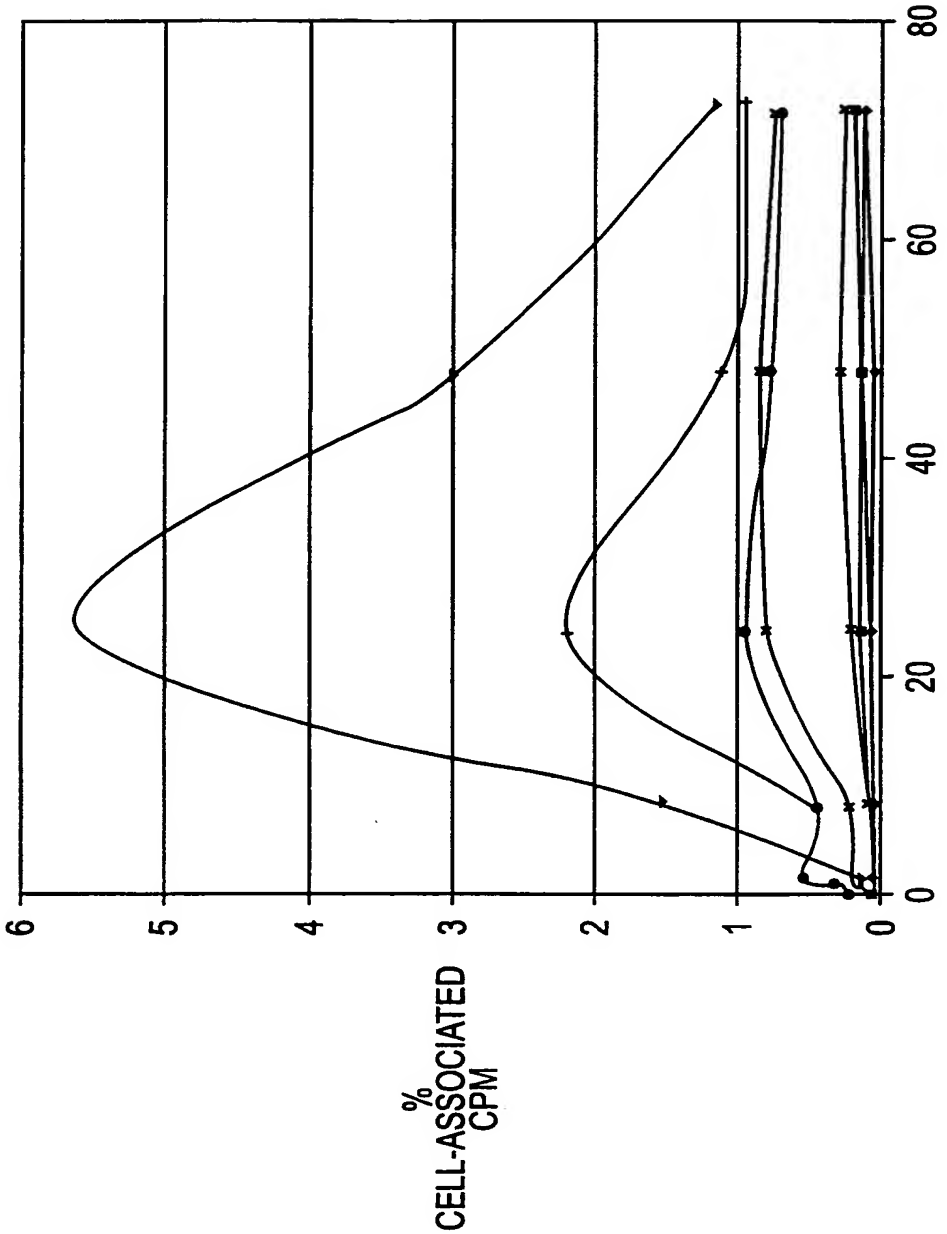


FIG. 8